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STUDIES ON BOVINE MASTITIS

1.—THE BACTERIOLOGY OF MASTITIS.

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INTRODUCTION.

IN the course of work on mastitis in bovine animals which was started in this Institute in the early part of 1928, the predominating bacterial species have been isolated from a number of samples of udder secretion which have been forwarded by veterinary surgeons at our request or which have been collected by ourselves. We wish to take this early opportunity of acknowledging our indebtedness to the many members of the veterinary profession who have assisted by sending material. The present article contains a survey of the bacterial types encountered, with more particular reference to streptococci, which are well known to be the most prolific cause of mastitis among cows in milk. In later publications from this Institute it is hoped to deal in greater detail with some of the bacteria which are more rarely associated with mastitis, as well as with other aspects of the general problem.

Bacterial Types Associated with 113 Cases of Mastitis.

The predominating types met with were as follows:—

Streptococci	..	..	..	..	..	82
Hæmolytic	..	..	..	..	..	52
Non-hæmolytic or green-producing (<i>viridans</i> type)						30
Staphylococci	..	..	..	..	..	6
<i>B. pyogenes</i>	..	..	..	..	..	21
Gram-negative bacteria of <i>B. coli</i> or allied group	..					3
<i>B. necrosis</i>	..	..	..	..	..	1

The terms "hæmolytic" and "non-hæmolytic," as used throughout this paper, refer to the streptococcus types differentiated by J. H. Brown (1919) according to their growth in blood agar. Thus, the term "hæmolytic" is used with reference to the group of streptococci styled "beta-hæmolytic" by Brown, that is, deep colonies were surrounded by a clear zone of laked corpuscles. The non-hæmolytic forms corresponded for the most part to Brown's alpha type. In the latter case after short periods of incubation deep colonies in blood agar were surrounded by a greenish zone of methæmoglobinised corpuscles, and after continued incubation by an outer zone of varying width in which hæmolysis was more or less complete. A minority of the non-hæmolytic forms corresponded with the gamma type of Brown, in which growth in blood agar produces no obvious change in the surrounding corpuscles.

The above list may be looked upon as giving some general indication of the frequency with which the various bacterial types are associated with mastitis, because the material from which they were derived was unselected and was received from different parts of the country over a period of 18 months. However, the list should not be taken as comprehensive. It does not include such organisms as *B. tuberculosis*, because tuberculous mastitis does not come within the scope of this research, nor does it include other forms which may at times cause mastitis, such as actinomyces and possibly certain yeasts. As it stands, however, the table gives some indication of the multiplicity of bacterial types which in nature may produce pathological disturbance within the cow's mammary gland, and it also illustrates the complicated task confronting the investigator and the person whose duty it may be to carry out measures of control.

The above findings are similar in many respects to those of others. The earlier observers (Bang, Kitt, Guillebeau, and others) recognised that natural cases of mastitis might be caused by a variety of bacteria (Jensen, 1897).

Guillebeau, for example, isolated from 19 cases of "sporadic mastitis" streptococci, staphylococci, or Gram-negative bacilli.

Savage (1907-8) investigated 31 cases of mastitis from which organisms were isolated as follows: streptococci 21 times, staphylococci five times, *B. coli* once, *B. tuberculosis* once, while in three cases the cause was doubtful.

F. S. Jones (1918¹) examined milk from 81 animals suffering from mastitis with findings as follows: non-hæmolytic streptococci in 31, hæmolytic streptococci in 17, mixed hæmolytic and non-hæmolytic in two, micrococci in 24, *B. coli* in two, *B. lactis aerogenes* in one, and pleomorphic Gram-positive rods in four.

Hardenbergh and Schlotthauer (1927) studied the bacteria associated with 68 cases of mastitis occurring over a period of 2½ years in 65 animals belonging

to four herds. From 47 of these streptococci were isolated, 38 being of the non-hæmolytic and nine of the hæmolytic type; in seven cases staphylococci were regarded as the cause, and in six cases occurring in the same herd over a period of six months bacteria of the colon group were responsible.

I. STREPTOCOCCI.

The first important contribution to the bacteriology of bovine mastitis is that of Nocard and Mollereau in 1887. These authors described the cultural characteristics in simple media of a long-chained streptococcus which could be observed microscopically in secretion from the affected udder. Organisms of a similar appearance were encountered in several outbreaks of mastitis which were regarded as being of a contagious nature and considered by Nocard and Mollereau to belong to a definite type which they named *Streptococcus mastitidis contagiosæ*. This type may be viewed as identical with that termed *Streptococcus agalactiæ* by Guillebeau.

After the discovery of Nocard and Mollereau long and short-chained streptococci and diplococci were seen by several observers in the altered secretion of the udder, the shorter forms showing a marked tendency to intraleucocytic arrangement. On the basis of morphological differences of this nature certain earlier authors endeavoured to differentiate various forms of mastitis. Stäheli (1904), however, showed that such a distinction was not possible, and that length of chain was a variable characteristic and depended in nature upon such influences as body resistance and under cultural conditions upon the medium and temperature of growth.

Savage (1906-7, 1907-8) isolated a large number of strains of streptococci from the secretion of cases of clinical mastitis by cultivation on the surface of agar plates. An examination was made of their morphology and characters of growth in broth, and their effects on litmus milk and on various test substances. Although on the whole the reactions were somewhat variable, a type could be distinguished which possessed the following characters. Growth in plain broth occurred in the form of a coherent deposit leaving the supernatant medium nearly or quite clear. Microscopically the broth deposit was composed of long intertwining chains. Litmus milk was rendered acid and coagulated within 36 hours. Acid but no gas was produced from lactose and saccharose, occasionally from salicin, rarely from raffinose, and in no case from mannite or inulin. A similar organism could be isolated in many instances from the milk of cows with normal or practically normal udders, though even here the organism seemed to bear some relationship to mild pathological changes, such as ulcerated teats or slight induration of the gland. The similarity of this organism to that of Nocard and Mollereau was recognised.

In one case of mastitis Savage encountered a second type of streptococcus which was distinguished by its failure to produce acid from any test substance, and by producing very little acid and no clot in milk. The organism grew in broth in the form of long twisted chains, was pathogenic for guinea-pigs and mice on intraperitoneal inoculation, and is possibly similar to one previously described by Klein (1900) under the name *Streptococcus radiatus* (*pyogenes*).

Gminder (1912), following the lead of Schottmüller, used media containing blood with the object of differentiating pathogenic streptococci from saprophytic forms occurring in milk within the udder. The centrifuged deposits from 26 samples of mastitis secretion were smeared on the surface of blood agar and selected streptococcus colonies were transferred to various media. Strains from four samples only were hæmolytic, the remainder resembling the type "*viridans*" (Schottmüller). No relation could be noted between hæmolysis and the virulence of strains for mice. The general conclusion was that,

though blood media appear to be helpful, it is not possible by cultural methods to distinguish with certainty between saprophytic and pathogenic streptococci. Fermentation studies were not made.

F. S. Jones (1918) used horse blood agar poured plates as a means of isolating streptococci from cases of mastitis and in his series non-hæmolytic varieties predominated. The strains isolated were examined for their morphological characters and type of growth in broth, their action upon litmus milk and the common test substances, and their pathogenic power for rabbits. Morphologically the length of chain was variable. Characters of growth in broth were also variable, some strains producing a flocculent deposit with clear broth, others a moderate diffuse turbidity. All strains acidified and coagulated milk, except a few non-hæmolytic ones with which coagulation was partial. With one exception, all strains fermented glucose, lactose, and saccharose and failed to ferment mannite, inulin, and raffinose. The exceptional strain produced no acid from saccharose and little from glucose and lactose. Salicin fermenters predominated, since this substance was attacked by 34 out of 39 non-hæmolytic strains and by 19 out of 29 hæmolytic strains. Jones considers that a subdivision can be made in each of the two hæmolytic types on the basis of salicin fermentation.

Ayers and Mudge (1922) examined milk samples from 54 cows belonging to two herds. Some of the animals were suffering from mastitis, but the streptococci isolated from these resembled many of those isolated from clinically healthy cows. Of 100 cultures of streptococci obtained in this way 79 were considered to belong to a specific mastitis group, and 64 of these were beta-hæmolytic and 15 were described as non-hæmolytic (gamma type, Brown). The 79 strains showed the following characters in common: long chain formation in broth, acid and clot in litmus milk at 37° C. with partial reduction of the litmus, no change in litmus milk at 10° C., acid production in glucose, lactose, and saccharose, and no acid production in mannite, inulin, and raffinose. Twenty-six out of the 64 beta strains and eight out of the 15 gamma strains fermented salicin. Nine cultures belonging to a second group were not considered to be mastitis streptococci, partly because they acidified but failed to clot milk at 37° C., and also because they showed slight acid production in milk at 10° C. and fermented mannite, raffinose or inulin. The twelve remaining cultures were referred to a special group described as *Streptococcus acidominimus* owing to the fact that they did not lower the pH of media containing glucose, lactose, saccharose, or salicin below 6.2 to 6.4. These strains with one exception belonged to Brown's alpha group, and two of the twelve fermented mannite or inulin and produced no change in litmus milk at 37° C. They expressed no opinion as to the association of these twelve strains with mastitis.

Mejlbo (1924) examined the sugar fermentation reactions of 101 strains of streptococci isolated from milk samples of cows which had had mastitis or were suspected of having it. The streptococci isolated were regarded as those of mastitis. The strains were arranged in eight groups on the basis of fermentation results with 28 test substances. An examination of Mejlbo's results, however, shows that 95 out of the 101 strains fermented lactose and saccharose, and 71 of these showed no fermentation of mannite, inulin, or raffinose, 16 of the latter also failing to ferment salicin. Of the 101 strains 21 fermented mannite, and of these all but one fermented salicin, eight fermented inulin, and two fermented raffinose. At least 71 per cent. of Mejlbo's strains therefore belonged to a common group similar to that described by Savage and others.

Klimmer and Haupt (1926) isolated 44 strains of streptococci which corresponded to the type *S. agalactiæ* (Guillebeau) from cases of mastitis or from

cows suspected of mastitis. All strains were true to type in their action on litmus milk at 37° C., in failing to grow at 10° C., and in certain other respects, though three fermented inulin. The majority of the strains were alpha-hæmolytic and none was beta-hæmolytic.

In a more recent article Klimmer, Haupt, and Roots (1928) reported on the examination of 201 streptococcus strains isolated from apparently normal milk and from a few cases of mastitis in four herds by means of an agar plate medium containing saccharose and brom cresol purple. Of these strains 180 corresponded to the type *S. agalactiæ* (Guillebeau), and were mostly of the alpha type, not one being beta. The remaining 21 strains were isolated from a single cow affected with mastitis and, though giving the alpha type of hæmolysis, they could be clearly distinguished from the other strains. Thus, they produced acid slowly and without coagulation in litmus milk at 37° C., grew slightly at 10° C., showed some reduction of methylene blue in milk, gave a final pH 5.0 to 5.3 in lactose broth, and fermented saccharose and salicin, but not raffinose.

The above survey of the more important literature dealing with the streptococci of mastitis points to the existence of a prevailing type, the characters of which are as follows. Growth in broth occurs in the form of delicate flocculent masses composed of chains which are frequently long and convoluted. The medium remains clear or practically so. Litmus milk at 37° C. is acidified and coagulated within the first 48 hours, usually with some reduction of the litmus in the lower part of the tube. Acid production without gas occurs in glucose, lactose, and saccharose, and occasionally in salicin, but no change is produced in mannite, inulin, or raffinose. Strains may be either hæmolytic or green-producing, or more rarely may produce no obvious change when growing in blood agar.

There is little doubt but that this was the type described by Nocard and Mollereau. It was certainly the type most commonly found by Savage, F. S. Jones, Ayers and Mudge, Mejlbo, and Klimmer and his associates; Savage and Mejlbo, however, did not use blood media. That our observations in the main closely parallel those of the authors cited will be evident when the results which follow are considered.

Technique.

In practically every case the material was examined on the day it was taken or on the following day.

A smear of the altered secretion or of the centrifuged deposit was examined microscopically in the hope that some judgment might be formed of the degree of dilution required to furnish isolated colonies. Starting with one drop or rarely over 0.5 c.c. of the secretion, serial dilutions were sown in three to five 5 per cent. ox blood agar poured plates and incubated at 37° C. After 24 to 48 hours the type of hæmolysis if present was observed by the naked eye and under a low power of the microscope, and two or three of the predominating colonies were picked from the most suitable plate to tubes of serum broth. If the cultures from the colonies were pure, as was usually the case, they were used for inoculating mice and for sowing tubes of plain broth, litmus milk (for incubation at 37° C., and at 15° to 18° C.), fermentation media, 1.0 per cent. glucose broth, and plain broth containing 1.0 per cent. sodium hippurate.

The plain broth medium was observed at the 24th hour to determine the nature of the growth and its microscopic characters. Litmus milk at 37° C.

was examined on the first, second, and fifth days. Litmus milk at 15° to 18° C. was examined at intervals until about the 20th day. The fermentation medium consisted of 1·0 per cent. peptone water enriched with approximately one-tenth of its volume of plain broth, to which after sterilisation horse serum and test substance were added up to concentrations of 1·0 per cent., the final pH being 7·2 to 7·4. Readings of the H-ion concentration were taken colorimetrically after five days at 37° C. The final H-ion concentration in 1·0 per cent. glucose broth was also noted after five days at 37° C. Hydrolysis of sodium hippurate to glycocholic and benzoic acid was sought according to the recommendations of Ayers and Rupp (1922), by growing the organism in peptone hippurate broth for five days at 37° C., followed by addition of 12 per cent. acidified ferric chloride solution. Results were not considered positive unless a heavy precipitate appeared on the addition of ferric chloride, and where the first result was negative the test was repeated at least once. In some cases slight precipitates which occurred on addition of the reagent were examined for the presence of benzoic acid with negative results. The production of hæmolsin was also investigated by growing the organism in 15 per cent. horse serum broth for 14 hours at 37° C., after which 1·0 c.c. of the whole culture was added to 0·5 c.c. of 5 per cent. suspension of washed ox blood corpuscles and the mixture incubated in a water bath at 37° C. for two hours.

Cultural and Fermentation Results.

Of the 82 strains of streptococci isolated 52 were hæmolytic in blood agar, and 27 were of the green-producing variety, while with three strains the blood corpuscles in the neighbourhood of the colonies remained unaltered.

Hæmolytic Strains. Of the 52 strains belonging to this group all but one (No. 38) could be regarded as belonging to a common type, provided that minor cultural differences such as might occur in broth were disregarded. The strains in this group produced a flocculent type of growth in broth, often composed of chains of considerable length. Litmus milk was rendered strongly acid and firmly coagulated within 24 to 48 hours, with subsequent reduction of the indicator in the lower third or half of the clot. Later the clot contracted slightly and expressed a clear whey. Glucose, lactose, and saccharose were fermented by all strains, and mannite and inulin by none. 46 strains fermented salicin, though in eight cases the production of acid was either low (final pH 5·6 to 6·6) or inconstant, or did not appear until the strain had been in stock for a year. 30 strains were tested for fermentation of raffinose with negative results.

Thirty-four of the 52 strains which were hæmolytic in blood agar produced recognisable amounts of hæmolsin when cultivated in 15 per cent. horse serum broth.

Hippurate splitting was common in the hæmolytic group. Of 42 strains tested 37 gave an unmistakably positive reaction with ferric chloride. The five remaining strains failed to split hippurate. With regard to the final H-ion concentration in 1 per cent. glucose broth, 32 hæmolytic strains out of 36 reduced the pH below 5·0, and with three of the remaining strains the final pH did not exceed 5·1.

The only strain which presented important deviation from type

belonged to the hæmolytic group. This strain (No. 38) was exceptional in that it failed to ferment lactose, although in other respects its fermenting powers were similar to those of the remaining strains in the hæmolytic group. This strain was tested several times shortly after isolation in litmus milk at 37° C., and produced no change beyond slight reduction of the litmus; after several months' storage it rendered milk acid without coagulation. It produced slight bleaching only in litmus milk at 15-18° C. It failed to split hippurate and gave a final pH in 1 per cent. glucose broth of 5.0.

Non-hæmolytic Strains. The characters of the 30 non-hæmolytic strains were more variable but for purposes of description they may be placed in two groups.

The first group was composed of 14 strains differing in no essential respect from the hæmolytic strains except in their effect upon blood. The second group of 16 strains differed in that the ability to acidify milk was decidedly more feeble and coagulation was usually incomplete or occasionally entirely absent. In most cases the feeble action in milk could be correlated with a lowered capacity to utilise glucose, lactose, and saccharose, and with complete failure to utilise salicin. The presence of 3.0 per cent. serum in the medium failed to bring about fermentation of salicin or mannite.* No raffinose fermenters were met with and no strain produced hæmolysin in serum broth.

Hippurate splitting was less common among the non-hæmolytic strains. Thus, of 28 strains nine failed to split appreciable amounts of hippurate.

With twelve out of 24 non-hæmolytic strains the final pH in 1 per cent. glucose broth did not fall below 5.0, and of these twelve strains eleven produced only partial clotting in milk, and nine of the twelve strains failed to split hippurate. In the case of five other strains the results were variable, though even with these the tendency was for the final pH to be above rather than below 5.0.

In considering the above results it is evident that, apart from their action in blood media, the hæmolytic and non-hæmolytic groups of mastitis streptococci possess many properties in common. These are summarised in Table I.

In their cultural and fermentation reactions practically all the hæmolytic strains and about one-half of the non-hæmolytic strains fall into a single group. Within the hæmolytic group the characters of the strains were on the whole fairly constant, though there were variations in the degree of hæmolysis in blood agar, and one-third of the number produced no recognisable amount of hæmolysin in serum broth. In the non-hæmolytic group, on the other hand, there was a higher degree

* Failure to ferment mannite and inulin in the alpha group is important because there may occasionally be encountered in milk drawn from the udder a small non-hæmolytic streptococcus which ferments both these substances. We have no satisfactory evidence that this type is associated with mastitis.

TABLE I.
REACTIONS OF MASTITIS STREPTOCOCCI.

Type	Total No. of Strains	Growth in Broth	Litmus Milk	Fermentations of							Hip- purate splitting	Final pH in 1 per cent, Glucose Broth		Hæmolytin production in 15 per cent. Serum Broth	
				Glucose	Lactose	Saccharose	Salicin	Mannite	Inulin	Raffinose		Below 5.0	5.0 or above		
Hamolytic	52	Flocculent deposit with clear supernatant	Acid clot, partial reduction	+	+	+	46+ 6—	—	—	—	(30)—	(42) 37+ 5—	(36) 32	4	34 + 18 —
Group I Non-hæmolytic	14	Do.	Do.	+	+	+	13+ 1—	—	—	—	(13) 11+ 2—	(11) 10	1	—	—
Group II.	16	Slight diffuse turbidity	Acid, clot incomplete or absent	+	+	+	6+ 10—	—	—	—	(15) 8+ 7—	(13) 2	11	—	—

Figures in brackets refer to the number of strains tested.

of variability. Approximately one-half of the strains in this group were distinguished by failure to produce high acidity in media containing fermentable carbohydrate, by failure to cause rapid and firm coagulation of milk at 37° C., and by inability to split sodium hippurate. These properties in general showed a pronounced tendency to run parallel; for example, strains which had no action on hippurate almost always failed to depress the pH of glucose broth below 5.0, and *vice versa*.

In these experiments there were included seven hæmolytic strains of human origin, received through the kindness of the National Collection of Type Cultures and of Dr. E. W. Todd. Four of these were derived from cases of scarlet fever, and two of the remainder from cases of broncho-pneumonia and puerperal septicæmia respectively. Although resembling hæmolytic mastitis streptococci in their effect upon blood, the seven human strains differed in that they did not split hippurate or coagulate milk and in failing to reduce the pH of 1.0 per cent. glucose broth below 5.1.

Pathogenicity for Laboratory Animals.

The general conclusion which may be gathered from the published works is that mastitis streptococci possess low pathogenicity for small laboratory animals, such as mice and rabbits. With a few exceptions our experience has been similar. Altogether, 40 hæmolytic strains and 23 non-hæmolytic strains were injected into mice intraperitoneally or subcutaneously in doses of 0.5 c.c. of 24-hour serum broth culture. On the whole, the hæmolytic strains were somewhat more pathogenic than the non-hæmolytic. Thus, of 145 mice inoculated with the hæmolytic strains, 48 or 33.1 per cent. died within seven days, while of 86 mice inoculated with the non-hæmolytic strains 17 or 19.7 per cent. died within seven days. The exceptional hæmolytic strain (No. 38) was among those which produced pathogenic effects in mice.

Many of the strains which proved to be fatal to mice were tested on rabbits by intravenous injection. Nine strains only (four hæmolytic and five non-hæmolytic) were found to be possessed of noteworthy pathogenic power and capable of killing within three or four days in doses of 2.0 c.c. or less of 24-hour serum broth culture. Two of the hæmolytic strains were exceptionally virulent, the minimum lethal doses being in the neighbourhood of 0.1 and 0.001 c.c. respectively.

Mice which did not die were killed on the seventh day, and in many cases smears or cultures were made from the heart blood, in the majority of cases with negative results.

Relationship of Hæmolytic and Non-Hæmolytic Forms of Streptococci to Mastitis.

From what has been said above, it may be gathered that streptococci which can be isolated from udder secretion in cases of bovine mastitis cannot be referred to a single well-defined group with common characteristics. There is beyond doubt a tolerably well-defined group which is associated with a large proportion of cases, *viz.*, that which corresponds with the *Streptococcus mastitidis* of Nocard and Mollereau. The strains

comprised in this group, however, differ in certain respects, and particularly in their action upon blood in blood agar plates. Many produce clear zones of hæmolysis around the deep colonies, while with others the deep colonies are surrounded by greenish zones due to the production of methæmoglobin.

The characters of the green-producing group are not so uniform as those of the hæmolytic group. A number of the former are distinguished by a lower activity in acidifying and coagulating milk, and in many cases this may be correlated with a lower production of acid in media containing glucose, lactose, and saccharose, and with failure to split salicin. These strains have a tendency to grow in the form of short chains in broth, and, consequently, cultures in this medium often show a slight diffuse turbidity as well as some deposit.

It may be assumed with some reason that all the types of streptococci represented by the strains isolated are at times causally connected with mastitis. In the present series, with two or three exceptions, the strains were derived solely from cases in which the secretion from the udder was visibly altered. By making a selection in this way it was hoped to exclude from the collection bacteria which might be regarded as normal inhabitants of the udder. Care was invariably taken to pick colonies of the predominant type. There was usually little difficulty in doing this because the organisms were present in pure or practically pure culture, and in such large numbers that well-isolated colonies were confined to plates 3 to 5 in the series.

The question now arises whether some or all of the types represented should be regarded as pure parasites or more properly as normal inhabitants of the udder which for some reason or other have temporarily increased in numbers. This question cannot be answered categorically by stating that streptococci possessing the characters mentioned are never encountered in the "normal" udder, because it is now well recognised that such streptococci may be encountered at times in milk samples from udders in which there is no obvious pathological change, and in which even laboratory examination may fail to reveal distinct abnormality. This cannot be held to indicate, however, that these streptococci are normal inhabitants of the udder, since there is no denying the facts that in most quarters in which these streptococci can be demonstrated pathological disturbance does arise sooner or later, and that the majority of clinical cases of mastitis are associated with one or other of the streptococcus types under consideration. Moreover, experience in this Institute has shown that in cases where repeated laboratory examination of the milk has failed to show any sign of pathological reaction these streptococci cannot be isolated. The evidence therefore points to the parasitic nature of these organisms.

It does not follow that all the types of streptococci isolated from these cases of mastitis are equally parasitic, or that the disease condition they produce is always of similar severity. In the present series hæmolytic forms predominated. Some other observers, however, have reported a preponderance of non-hæmolytic forms. The difference probably depends upon the origin of the material, whether this is

derived from one or two herds or from a larger number of herds distributed over a wider area, as well as upon the varying severity of disease type. Thus it is known that among streptococcus infections of other animals, and particularly of man, those caused by streptococci of the hæmolytic type are usually more severe or acute than those caused by non-hæmolytic forms. This is in accordance with the belief that the hæmolytic forms are more highly specialised and more adapted to a parasitic existence than non-hæmolytic streptococci, which tend to be more saprophytic in nature. By analogy, therefore, it might be expected that hæmolytic streptococci would be encountered in larger numbers in herds in which the clinical type of mastitis is of greater severity. We have endeavoured to collect some evidence relating to these points from the clinicians who were in touch with the cases. The information obtained lends support to the opinion that mastitis due to the hæmolytic type is usually more severe in its course, and produces more profound disturbance within the gland than mastitis due to non-hæmolytic streptococci. The experience which is being gained at this Institute in the course of routine examination of herds is also in agreement with this opinion. On clinical grounds, however, it would probably be impossible to make any clear distinction.

SUMMARY.

Of 113 unselected cases of bovine mastitis, material from which was received over a period of eighteen months, 82 were due to streptococci. Judging by their cultural and biochemical characters, the strains isolated belonged to a fairly well-defined type, similar to that which has been identified by previous authors, and to which the name *Streptococcus mastitidis bovis* may be applied. There were outstanding differences however, in the action of the strains in blood agar, 52 or more than half the series being hæmolytic, and the majority of the remainder being non-hæmolytic forms of the green-producing or *viridans* type. With one exception, the hæmolytic strains showed considerable uniformity in their characters, while among the non-hæmolytic forms there was greater variability. Approximately one-half of the latter could be distinguished by their appreciably lower acid production from glucose, lactose, and saccharose, and by lower activity in acidifying and clotting milk.

The majority of the mastitis strains were capable of hydrolysing sodium hippurate and of increasing the hydrogen-ion concentration of 1·0 per cent. glucose broth to a final pH value of 4·4 to 4·8. These two properties showed a tendency to run parallel, but they were not uniform within the two groups. Thus, of the 42 hæmolytic strains 37 were capable of splitting hippurate, and 32 out of 36 gave a final pH in glucose broth of less than 5·0. Among the non-hæmolytic strains nine out of 28 failed to split hippurate, and twelve out of 24 gave a final pH of 5·0 or over in glucose broth. Practically the whole of the strains giving a final pH in glucose broth higher than 5·0 and not splitting hippurate were included in that section of the non-hæmolytic group which caused slow and partial clotting of milk. In these respects

they resembled seven strains of streptococci from human sources, including four scarlet fever strains, which were introduced for comparison.

The general level of pathogenicity of the mastitis strains was low, though two hæmolytic strains were found to be exceptionally virulent and capable of killing rabbits by intravenous inoculation in doses of 0.1 c.c. and 0.001 c.c., respectively, of a 24-hour serum broth culture.

II. STAPHYLOCOCCI.

Staphylococci were regarded by the earlier workers as the cause of certain of the clinically more acute or purulent forms of mastitis, or of the so-called "parenchymatous mastitis," as opposed to the more slowly progressive "catarrhal mastitis" which was caused by streptococci (Jensen, 1897).

Guillebeau spoke of "galactococci" as the cause of mastitis, but from the description it is evident that he was dealing with certain varieties of staphylococci.

Sven Wall (1908) gives a good description of the morbid appearances of the udder in two cases which were due to staphylococci, and in which the inflammatory changes had led to necrosis. According to this author, cases are always acute, and may be fatal.

Savage (1907-8) described five cases, of which only one was severe clinically, and in these staphylococci were regarded as the possible cause. Savage considered the evidence of association of staphylococci with these cases as very incomplete.

F. S. Jones (1918) described 28 strains of micrococci (staphylococci), 23 of which liquefied gelatine at 22° C. Of these, 21 fermented glucose, lactose, saccharose, and mannite, and eleven inulin and raffinose also. The cases of mastitis were usually mild, a few only being acute.

Mastitis due to staphylococci has not received as much attention as the more common streptococcus form. Staphylococcic mastitis, however, is of importance, because, although apparently less common, cases at times assume a very acute course, and may even be fatal.

During the period covered by this work we have received udder secretion from six cases of mastitis in which staphylococci were present in such predominating numbers as to suggest strongly that they were the cause of the disease. In eight other cases the history suggested staphylococcic mastitis, but for some reason a satisfactory diagnosis could not be made.

The six cases could be classified clinically as subacute or acute, and most of them were reported as having occurred within the first few days after calving, either during the winter or the summer. In some instances it was stated that similar cases had occurred previously in the herd. The usual signs of constitutional disturbance were present, such as loss of appetite and rise of temperature, and these symptoms were referable to pathological changes in the udder, of which one or more quarters were found to be considerably swollen and tense, hot, and painful. Sometimes the disease process appeared to have started in one quarter and to have extended later to other parts of the udder. Two of the six cases died. In one of these the local changes were so severe that a portion of one-quarter became "gangrenous," and death

occurred two days after the onset and four days after calving. A third animal had to be slaughtered. In this case the udder was examined within 24 hours of death, and the most severely affected quarter showed on section irregular necrotic areas with deeply congested edges. Microscopic examination of sections from these areas showed dense masses of staphylococci. In the remaining cases it was ascertained that the acute local symptoms gradually subsided and the animal recovered. The characters of the secretion varied with the severity of the attack. In the milder cases the fluid resembled thin milk or whey, and contained small clots; in the more acute cases it was thin and watery and deep reddish-brown in colour, like hæmolysed blood. The secretion was usually excessively alkaline in reaction, and had no special odour.

Diagnosis was effected in all these cases by centrifuging the secretion and sowing successive dilutions of the deposit into blood agar. Usually the first one or two plates were crowded with colonies, and the medium partially or completely hæmolysed. Subsequent plates showed isolated deep colonies which were surrounded by zones of incomplete hæmolysis up to about 8 mm. in diameter.

The main characters of the staphylococci associated with these cases were as follows. In broth they produced a moderately dense uniform turbidity composed of cocci of rather small size arranged for the most part in small clumps. On the surface of agar, solid serum, or potato, colonies were dirty-white or pale fawn in colour. The organism grew in gelatin stab at 15°-18° C., and by the third week two-thirds of the medium was liquefied. Litmus milk was rendered acid within the first 24 hours and firmly coagulated, the clot showing considerable contraction with expression of clear whey. Acid was produced in glucose, lactose, saccharose, and mannite, but not in salicin or inulin. Broth cultures were usually fatal within three or four days to mice when injected intraperitoneally in doses of 0.1 to 0.2 c.c., and staphylococci could be recovered from the heart blood. One strain produced fatal results in rabbits by intravenous inoculation in doses of 0.2 to 2.0 c.c., and the organism showed some tendency to localisation in the kidney.

SUMMARY.

A bacteriological examination has been made of material from six cases of mastitis which were associated with the presence of staphylococci. The clinical course of the disease was more acute than is usual with streptococcus mastitis, and two of the affected animals died. In one case the local changes resembled those of gangrene.

The six strains of staphylococci isolated appeared to belong to the same group, being similar to one another in their cultural and biochemical characters and in their action upon blood in ox blood agar plates.

III. BACILLUS PYOGENES.

Much has been written on the subject of so-called "summer mastitis," and the characteristic features of the diphtheroid organism,

B. pyogenes, which is the most frequent cause, have been described repeatedly. References to the literature are given by Karmann (1928) among others, and more recently in a dissertation by Rolle (1929).

During the course of this work *B. pyogenes* has been isolated by the blood agar plate method from samples of udder secretion from 21 cases of mastitis. With one exception all the specimens from which *B. pyogenes* was isolated were received during the six months from June to November.

It is well known that pyogenes mastitis is met with far more frequently in dry cows than in cows in milk. In twelve cases out of sixteen of the present series the first lesions were noticed in the udder from a few days to a month before calving. In another case the subject was a two-year-old maiden heifer at grass. In most animals one or more quarters of the udder were reported to be enlarged and firm, and in seven there were constitutional symptoms, including in two or three instances swelling of the hock joints. One animal died, and in most of the remainder the affected quarter was reported to have lost its function. In more than half the animals the course of the condition was chronic, and in some cases abscesses which had formed in the udder tissue had broken through the skin. In one herd three animals were affected at the same time.

The characters of the secretion varied from a greyish or yellowish whey-like liquid containing clots to a thick purulent fluid with a putrid odour. Microscopically, the secretion usually showed enormous numbers of short, slender, partially Gram-positive bacilli, and also rounded forms resembling minute cocci. Blood agar plates sown with serial dilutions of the secretion showed in the first plates after 48 hours incubation large numbers of small colonies surrounded by narrow zones of hæmolysis. In all cases two or three of these colonies were picked to tubes of serum broth, and the growths from these transferred to solid serum slopes. Cultures were not accepted as those of *B. pyogenes* unless the solid serum was liquefied at the points where growth occurred.

Apparently, in the majority of instances *B. pyogenes* was present in pure culture. In one case, however, hæmolytic streptococci were present at the same time; in two cases numerous colonies of staphylococci appeared on the first two plates in the series, and in one case microscopic examination of the pus showed a filamentous organism which could not be cultivated, but which was considered to be *B. necrosis*.

SUMMARY.

B. pyogenes was the organism most numerous present in samples of udder secretion from 21 cases of mastitis. With one exception all of these were observed during the second half of the year, and, in agreement with the common experience, nearly all the cases occurred in dry cows shortly before calving.

IV. COLIFORM BACTERIA.

Gram-negative bacilli isolated from cases of "parenchymatous mastitis" by Guillebeau and others were considered by Jensen to belong to the *B. coli* group (Jensen, 1897).

Savage (1907-8) described an acute case of mastitis accompanied by diffuse swelling of the quarter and some general disturbance. Large numbers of Gram-negative organisms identified as *B. coli* were present, and streptococci could not be isolated.

Sven Wall (1908) described the biological characters of Gram-negative bacilli which had been associated with mastitis and identified as *B. coli* or as *B. lactis aerogenes*. Clinically the cases referred to did not tend to be chronic, though they were seldom acute and rarely fatal.

Klimmer (1909) gives an account of the characters of the Gram-negative bacteria which had been isolated from cases of mastitis.

Zwick and Weichel (1910) described 21 cases of acute parenchymatous mastitis due to Gram-negative bacteria, and of the 21 strains isolated 19 were regarded as *B. coli*. The great majority of the cases ended in resolution, though milk secretion from the affected quarters was greatly diminished or completely arrested until the succeeding lactation.

Gilruth and McDonald (1911) investigated thirty cases of severe but non-fatal mastitis in a herd of forty cows. From many of these Gram-negative bacilli were isolated, the characters of which were said to resemble those of *B. lactis aerogenes*.

Jones (1918) described two cases due to *B. coli*. In one the secretion contained fine white flocculi, and in the other case, which was acute, the secretion resembled hæmolyzed blood. He also described a chronic case of mastitis in which the secretion was watery and flocculent and from which *B. lactis aerogenes* was isolated.

Hardenbergh and Schlotthauer (1927) encountered six cases of mastitis due to *B. coli* in one herd within a period of six months. The onset was sudden, and there was marked systemic reaction with greatly diminished milk secretion from the unaffected quarters. The affected quarters were firm and sensitive, and were ultimately lost. The secretion at first consisted of clear fluid with flakes of curd, and was transformed within a few days to thick fetid pus.

We have had an opportunity of making a bacteriological examination of three samples of udder secretion in which Gram-negative coliform bacteria were present in pure or almost pure culture.

Case I was a severe one in a first-calf heifer immediately after calving. The right hind quarter was a little hard and painful, the secretion from it consisting of a yellowish excessively alkaline watery fluid containing clots and possessing a sickly odour. The veterinary surgeon noticed that the secretion contained bubbles of gas when it was drawn from the teat. The animal was very ill for several days, but recovered with loss of the affected quarter. A very large number of hæmolytic colonies composed of short rods appeared in blood agar plates sown from the deposit.

Case II was mild clinically and rapidly recovered. The secretion was watery, excessively alkaline in reaction, and one quarter of its volume was made up of small curds. About 800 colonies with narrow hæmolytic zones grew in blood agar plates sown with 1 c.c. of the secretion, and very few colonies of any other type were present.

In Case III there was mild disturbance in two quarters of the udder just after calving. The secretion was a greyish-yellow thick liquid, normal in reaction. One drop plated in blood agar showed innumerable hæmolytic colonies.

The characters of these three strains are set out briefly in Table II, in which there are also included details of two Gram-negative cultures isolated from cases of mastitis in Palestine and forwarded through the kindness of Mr. S. J. Gilbert, M.R.C.V.S.

A survey of the table shows that the characters of the five strains are diverse, and it is not possible to recognise any well-defined group. The strains from Case I and Palestine No. 1, however, may be regarded as belonging to *B. coli* group, and that from Palestine No. II is somewhat similar except for its action on methyl red. The strain from Case II is sharply distinguished by its failure to clot milk, by liquefying gelatine, and by failure to ferment lactose and mannite. The culture obtained from Case III was highly proteolytic, and, though aberrant in a few of its reactions, it appeared to belong to the proteus group of bacteria.

SUMMARY.

Gram-negative coliform bacteria of diverse characteristics were found to be associated with three cases of mastitis.

V. BACILLUS NECROSIS.

Jenson (1897) considers that mastitis caused by *B. necrosis* arises as the result of embolism from primary necrotic lesions in other parts of the body, e.g., the mouth cavity, digestive tract, or skin, particularly from the skin round the claws.

Sven Wall (1908) states that necrobacillosis of the udder is very rare and that he himself never saw a case.

The comparative rarity of lesions in the udder due to *B. necrosis* gives added interest to the following case in a six-year-old cow.

Lesions were present in the left fore quarter only, and at the first examination this portion of the udder was uniformly enlarged and firm, but not sensitive to pressure. Thick ropy muco-pus could be drawn from the teat, and this material on standing in a tube expressed a small quantity of whey-like fluid. Smears of the pus showed microscopically large numbers of slender, Gram-negative bacilli, very variable in length but with a preponderance of long forms. The animal showed some systemic disturbance, and during the few preceding weeks had lost condition. The right knee joint was enlarged, and could not be flexed without great pain. An abscess formed in this situation later and burst, and the purulent discharge when examined microscopically showed the presence of large numbers of bacteria morphologically similar to those seen in the udder secretion. The animal recovered, but six months later the left fore quarter was considerably atrophied and had lost its function.

Several attempts were made without success to cultivate the organism from the original material on the surface of solid media—serum agar, blood agar, and solid serum. Such media placed in an anærobic jar remained apparently sterile, though occasionally there was microscopic evidence of growth. Slight growth took place in deep

TABLE II.

Strain from	Motility	Blood Agar	Neut. Red Broth	Potato	Litmus Milk	Gelatin	Indol production	Gluc.	Lact. Sac.	Sal.	Man.	Dulc.	Imulin	Pathogenic for	Reactions to Methyl Red and Voges-Proskauer	Remarks
Case I	Actively motile	Hæmolytic	Yellow	Colourless or very pale fawn	Acid, clot, gas	Growth on stab, no liquefaction	+	AG	AG	O	AG	AG	O	Guinea-pigs and mice	MR + VP —	
Case II	Actively motile	Hæmolytic	Yellow	Abundant, pale fawn	No acid or clot, slow and partial digestion	Upper 1/3rd of tube liquefied after 10 days	—	AG	O	Slight G	O	O	O	Mice only, not for guinea-pigs	MR + VP —	
Case III	Actively motile	Hæmolytic	Red	Pale fawn	Slight acid, no clot to 7 days	Liquefied in 2 days	+	AG	AG	AG	AG	O	O	Not pathogenic	MR + VP —	Also liquefied solid serum
Palestine No. I	Non-motile	Non-hæmolytic		Abundant, moist, white	Acid, clot, gas	No liquefaction	—	AG	AG	AG	AG	O	O	Mice slightly, not for g. pigs		
Palestine No. II	Actively motile	Non-hæmolytic	Yellow	Slight, colourless	Acid and clot after 5 days' incubation	No liquefaction	+	AG	Slight G	AG	AG	O	O	Guinea-pigs and mice	MR — VP —	

glucose agar shake media. In liquid media, such as glucose broth, under anærobic conditions growth failed to appear unless the medium contained pieces of tissue. The only medium which was found to be entirely satisfactory was liver broth under a layer of vaseline and containing pieces of cooked liver. The addition of 1·0 per cent. glucose greatly increased the abundance of growth. After 24 hours' incubation there was production of gas, the broth was slightly turbid, and there was a small white deposit. Cultures gave off a peculiar, unpleasant odour. Microscopic examination showed long slender filaments, staining evenly with thionin, and also a smaller number of broader forms with more deeply staining granules inset. On morphological and cultural grounds the organism appeared to be identical with *B. necrosis*.

A rabbit inoculated subcutaneously with a little of the original material developed a local abscess which had reached the size of a pigeon's egg by the 28th day, when the animal was killed. The contents of the abscess were yellowish-white in colour, semi-solid in consistence, and contained slender bacilli similar to those in the original material. A guinea-pig and two mice inoculated with the original pus showed no evidence of infection.

Further animal inoculations were made with a 24-hour glucose liver broth culture. A rabbit which received 1·5 c.c. subcutaneously on the inner side of the thigh died on the fourth day, with extensive œdema and crepitation at the point of inoculation. On removal of the skin the subcutaneous tissues were found to be infiltrated with blood-tinged liquid containing gas-bubbles, and the superficial layers of the muscles to a depth of 3 or 4 mm. were blanched as the result of necrosis. No lesions were visible in the internal organs. Slender bacilli and filaments, some of which were beaded, were present in smears from the local lesion.

A guinea-pig inoculated subcutaneously with 1·0 c.c. culture showed no change beyond slight œdema at the point of inoculation. Subcutaneous inoculation of 0·5 c.c. amounts in mice caused death within six days. At the point of inoculation there was a sharply-defined, yellowish-white, necrotic lesion, involving the skin, subcutaneous tissues, and muscles. In one instance the lesion extended round the body from the point of inoculation on the back to the umbilical region, and involved the abdominal muscles and spleen. Thus, this strain of *B. necrosis* was found to be typical of the species in being pathogenic for rabbits and mice, but not for guinea-pigs.

This is the only case in which we have isolated and identified *B. necrosis*. One other case of mastitis was encountered in which an organism morphologically similar to *B. necrosis* was present in the pus. Owing to the presence of large numbers of *B. pyogenes*, however, it proved to be impossible to obtain pure cultures.

SUMMARY.

A description is given of a case of mastitis caused by an anærobic organism identified as *B. necrosis*.

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NEPHRITIS IN ANIMALS.

By Sir JOHN MCFADYEAN.

(Continued from page 154.)

As shown in the previous article, there occurs in the dog a form of chronic nephritis in which multiple small cysts are formed by dilatation of straight tubules in the medulla. As a rule, in these cases the cortex remains free from cysts, but Fig. 1 represents an exception, in which nearly every Malpighian had been converted into a cyst. In this case the medulla showed the chronic interstitial changes associated with destruction of tubules described in the previous article, but, as in the cortex, all the cysts were of microscopic size, and they were less numerous than usual.

There appears to be no reason to doubt that in such cases the cyst formation in the cortex results from the obstruction to the free flow of urine in the tubules of the medulla.

Fig. 3 shows a rare condition in which extreme degeneration of the

epithelium of the convoluted tubes and calcification of their walls had occurred. This was also a case of chronic interstitial nephritis in an old dog, with the usual medullary lesions. Cortical cysts resembling those in Fig. 1 were also present, but less numerous, and in some of these the calcification involved the Bowman's capsule which formed the wall of the cyst, and even the atrophied glomerulus.

One or more large cysts may be present in the cortex in cases of nephritis (Fig. 4), but also in otherwise apparently normal kidneys.

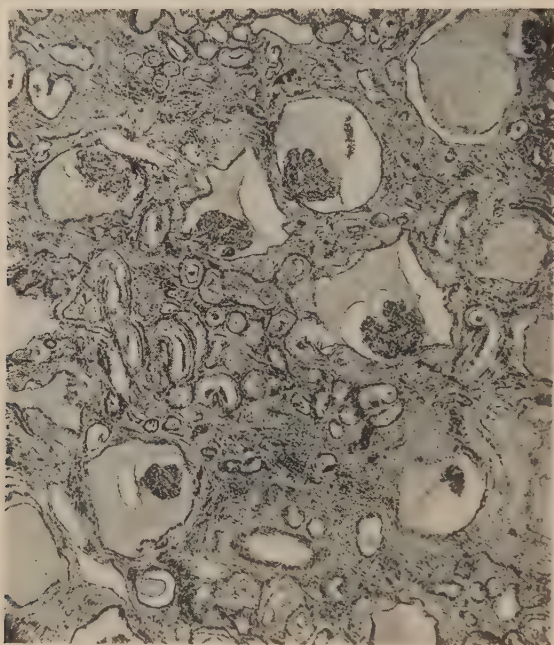


FIG. 1.

Multiple microscopic cysts in the cortex in a case of chronic interstitial nephritis in a dog.

Acute Interstitial Nephritis.

The cases of nephritis that fall to be included under this heading are those in which the primary, and, throughout the course of the disease, the predominant, alterations in the renal tissue occur in the interstices of the labyrinth—that is, between and around the convoluted tubules and around the malpighian bodies; and, of course, the alterations must be of a kind that indicates an acute inflammatory origin. Such changes are always present in the shape of congestion and accumulation of leucocytes, a most exclusively polynuclear, in the cortex in cases of embolic nephritis caused by streptococci and other pyogenic bacteria, but as a rule in cases classed under the above heading the intertubular cells are of the lymphocyte type, or at any rate mononuclear. These intruded cells may occur in the form of separate foci throughout the cortex (disseminated), or the cellular infiltration

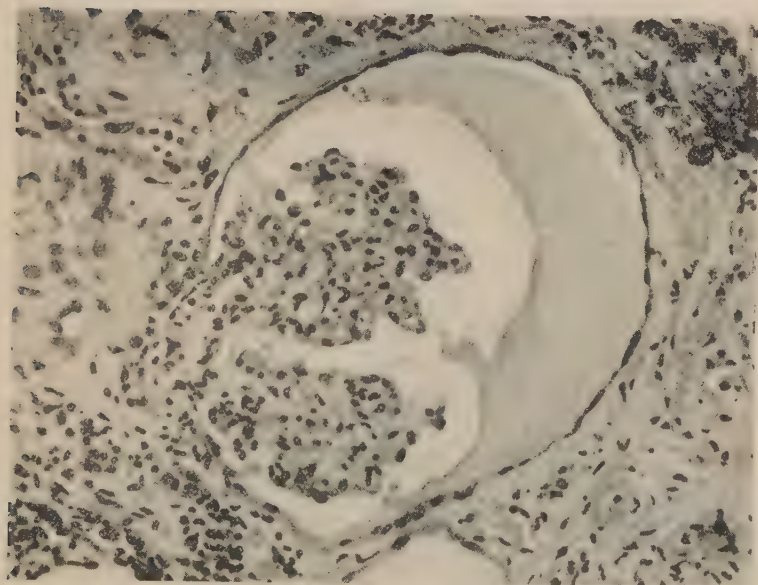


FIG. 1.
From the same case as Fig. 1. Higher magnification of a cyst formed by dilatation of Bowman's capsule,

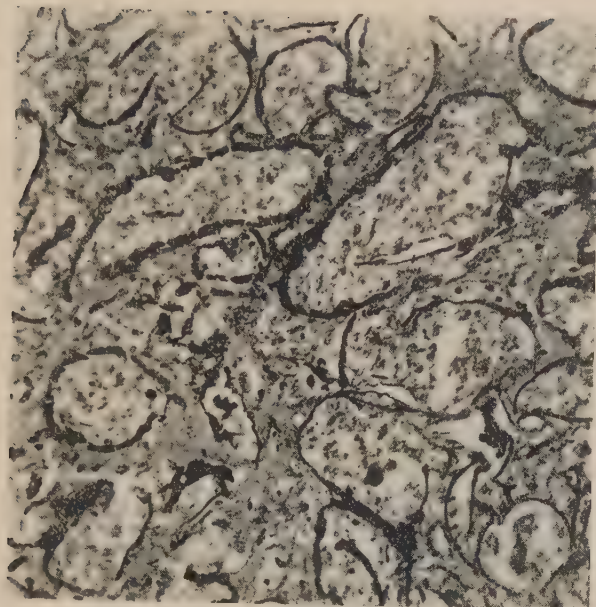


FIG. 3.
From another case of chronic interstitial nephritis in a dog. Shows thickening and calcification of the walls of the cortical tubules and extreme degeneration of their epithelium.

may be more or less diffuse. Congestion of the cortical vessels is seldom very pronounced, and the destruction of the tubules in the infiltrated areas lacks the rapid liquefying effect of purulent inflammations.

The condition is generally bilateral, and the kidneys may be moderately enlarged or normal in size. In the disseminated form the alterations in the cortex may escape detection with the naked eye, and when larger or diffuse they appear as partially decolourised areas in the labyrinth. At present there appears to be no certain knowledge regarding the cause, or causes, of this form of nephritis, but the distribution of the lesions and their usual focal nature make it not improbable that in many cases they have a bacterial origin.

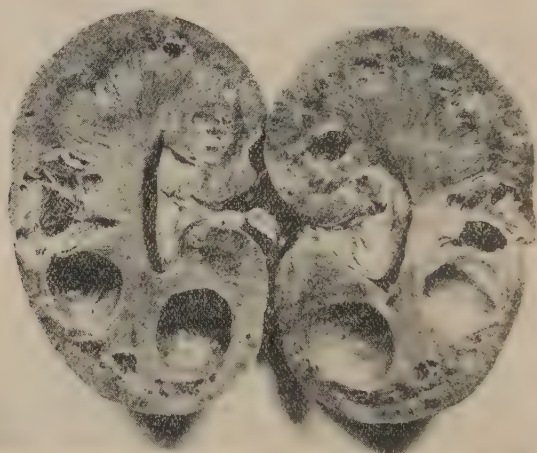


FIG. 4.

Divided kidney (slightly reduced) from a case of chronic interstitial nephritis in a dog.  Cysts present in all three strata of the kidney substance, but most appear to have had their origin in the boundary layer.

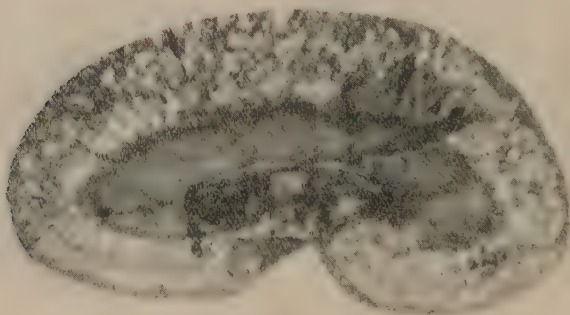


FIG. 5.

Kidney of dog, natural size. Disseminated acute interstitial nephritis.

The leucocytic infiltration of the cortex of the kidneys which occurs in leucocythæmia may be confounded with acute interstitial nephritis, and the risk is especially great in the dog, in which lymphatic leukæmia is far from rare. In that disease the nature of the areas of infiltration in the kidney may be recognised by the fact that at many places the position of the leucocytes is within distended capillaries (see Fig. 8, p. 238). When such lesions occur in the kidneys microscopic examination will show that they are also present in the liver.

Catarrhal, Tubular, or Parenchymatous Nephritis.

This has long been recognised in text-books as a special type of nephritis, in which the alterations mainly affect the renal epithelium, especially that of the tubules in the cortex. Morbid changes in these cells are very common, but the above designations should be reserved for those cases in which the tubular changes are the earliest and the most striking, and when that is done comparatively few cases deserving the name are encountered. There remains, however, the further point that extensive and, indeed, almost universal, fatty changes may involve the convoluted tubules without any of the vascular and tissue changes that are the head-marks of inflammation, and without these a case can hardly be regarded as one of nephritis.

The use of the term "catarrhal" in connection with nephritis is apt to recall the alterations that are commonly encountered in inflammation of mucous membranes, and especially proliferation of the epithelial cells, but a continued over-production of the lining epithelium of the uriniferous tubules appears to be a rare occurrence, though desquamation is common.

In spite of these facts, it may still be convenient to retain the term catarrhal for cases of nephritis in which alterations in the tubules are prominent, though not necessarily the first in point of time or importance. The alternative would be to make more frequent use of the term "mixed nephritis."

Catarrhal nephritis in the sense explained is common in the human subject, and acute and chronic forms of it are recognised. In the acute cases, apart from considerable active congestion, the most obvious alteration usually is swelling and loosening of the cells of the convoluted tubules, or actual necrosis and detachment, with consequent formation of epithelial casts.

In sub-acute and chronic cases the alterations are of the same nature, but more extensive, and more frequently accompanied by fatty degeneration and interstitial changes.

The precise etiology of many cases of catarrhal nephritis is obscure, but it is well known that various drugs or poisons (cantharides, turpentine, corrosive sublimate, uranium, and various metallic salts) may cause similar lesions. Suspected causes are bacteria or their toxins present in the blood in acute infective diseases. In the human subject sudden exposure to extreme cold has long been popularly regarded as a cause, but is alone inadequate to explain the lesions.

In the acute form the urine is always reduced in volume, highly

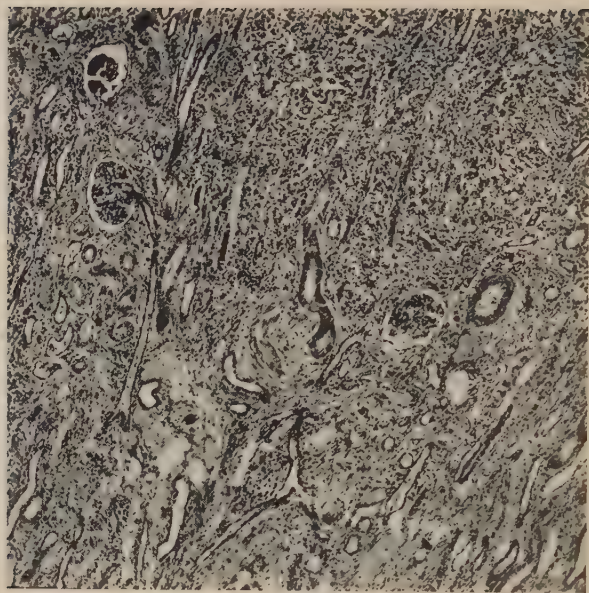


FIG. 6.

Section from kidney in Fig. 5. The upper half is from the deepest part of the cortex, and shows destructive infiltration with round mononuclear cells.

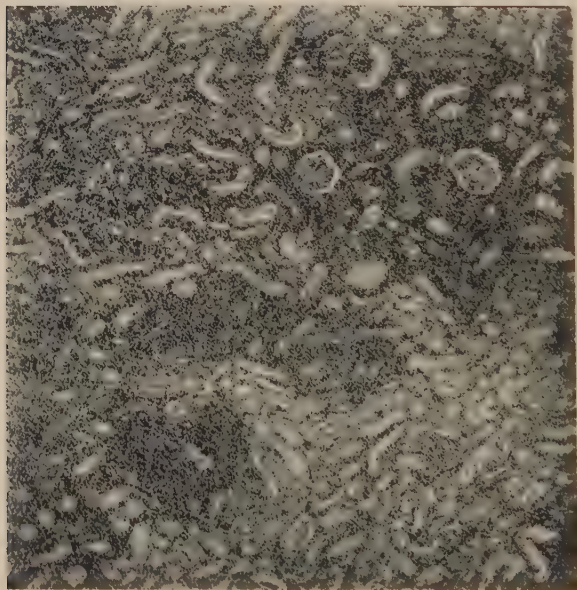


FIG. 7.

From another case of disseminated interstitial nephritis in a dog. Similar to Fig. 6.

albuminous, and visibly blood-tinged. In the chronic form it contains abundant suspended cells and cellular casts, and more or less albumen.

It is probable that complete recovery may occur in this form of nephritis when the cause has ceased to operate, the epithelial cells being regenerated from those that remain, but in chronic cases some interstitial changes are likely to occur and to be permanent.

Naked-eye examination is a very untrustworthy guide for the diagnosis of catarrhal nephritis, but it may be suspected when both kidneys are moderately enlarged, the capsules are not thickened or adherent; and the tissue of the labyrinth shows patchy pale discoloration. A uniform pallor of the cortex and congestion of the boundary layer and medulla is indicative of general fatty degeneration of the convoluted tubules without indications of inflammation—a condition frequently met with in the cat.

Glomerulo-nephritis.

Apparently the question whether there is a pure form of glomerulo-nephritis—that is, an inflammatory condition of the kidney affecting only the glomeruli—must have a negative reply. What is contended by most authors is that in man there occur cases of acute nephritis in which the whole or the great majority of the malpighian bodies are affected, and that, while other constituents of the kidney may become involved in the inflammation, the initial and most important alterations are in the glomeruli and their capsules. The acute nephritis which so frequently supervenes in cases of scarlet fever is, according to all authors, of this nature, but alterations of the same kind occur in other acute infective diseases, such as diphtheria, angina, acute rheumatism, and “trench fever.”

There is also recognised in man a chronic glomerulo-nephritis which leads to marked reduction in the size of the kidneys, with adhesion of the capsule, and irregularity of the surface of the cortex. The appearance of a kidney so affected may resemble that of a primary granular contracted kidney (see p. 142). The reduction in size affects the cortex mainly. There is an excess of interstitial connective tissue, mainly following the course of the interlobular arteries, and the tubules and malpighian bodies in it are found in various stages of atrophy.

Preparatory to considering whether acute glomerulo-nephritis occurs among the domesticated animals, it is necessary to note more precisely what are the alterations exhibited by the Malpighian bodies in the human cases.

At the outset it is to be noted that both kidneys are affected and to the same extent. They are appreciably enlarged and frequently, but not always, the Malpighian bodies are abnormally prominent, but without microscopic examination the existence of the nephritis might escape detection. Under the microscope, in the more acute cases, the loops of the capillaries of the glomeruli show a striking excess of leucocytes, and these may also be numerous in the capillaries immediately external to the capsules of the glomeruli. Accompanying or following



Fig. 8.

From a case of lymphatic leukæmia in a dog. Shows a thin crescent-shaped collection of degenerated epithelial cells inside the capsule of the Malpighian body. Great excess of leucocytes in the capillaries. Many other Malpighian bodies were affected in the same way. Epithelium of tubules swollen and many cells denucleated. Observe the resemblance of these to the cells of the crescent.

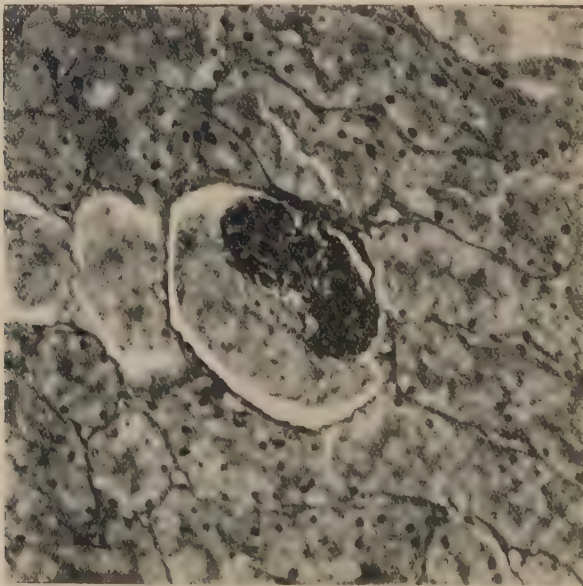


FIG. 9.

From the kidney of a cat, showing a collection of epithelial cells that appears to occupy more than half of the space enclosed by Bowman's capsule; the glomerulus is correspondingly reduced in size (see text).

these changes, the endothelium of the glomerular capillaries become swollen, and it is this swelling that ultimately dominates the picture (Aschoff). The glomeruli are now swollen to such an extent that hernia-like protrusions may extend through the neck of the tubule. Such swollen tufts show a great excess of nuclei, which belong in part to the capillary endothelium and in part to the leucocytes. The thickened capillaries have their lumen narrowed, and may in consequence contain little or no blood. The epithelium which invests the loops of the glomerulus shares in the swelling. The capsular space generally remains free from morphological elements, but in what is termed catarrhal glomerulo-nephritis the epithelium of both capsule and glomerulus may desquamate and proliferate, the cast-off cells accumulating to form a crescent-shaped mass between the glomerulus and its capsule. A fibrinous exudate may escape from the glomerular capillaries and be found in the capsular space or in the form of casts in the tubes. In the hæmorrhagic form of the inflammation blood may be found in and around the glomeruli and in the tubules.

Turning now to the question of the occurrence of glomerulo-nephritis in animals, one finds that, according to Henschen*, it is one of the most important forms in the horse, and occurs under different types. It is admitted, however, that most of the alleged cases in literature are of the sub-acute or chronic form, and that little is known regarding the alterations in the earlier stages of the inflammation. Particulars are given of four cases, designated respectively as representing the intra-capillary, the capillary thrombotic, the extra-capillary or capsular, and the exudative form.

The same author recognises a disseminated (herdförmige) type of glomerulo-nephritis in equines, but it is clear that in this he includes cases of embolic nephritis caused by the arrest and multiplication of pyogenic bacteria in the glomeruli, which, logically, have a good claim to the term glomerulo-nephritis, but by most authors are treated as a class apart. In fact, it appears to be not improbable that many of the cases of acute glomerulo-nephritis in the human subject are etiologically similar to the embolic pyæmic nephritis of animals, the difference in the effects being due to the difference in virulence of the respective bacteria (hitherto not identified as among the causes of glomerulo-nephritis of the human subject).

Apparently it was by including pyæmic nephritis as a type of glomerulo-nephritis that Henschen was able to regard the latter as one of the most important forms of nephritis in the horse.

The present writer is of the opinion that cases of the chronic form of glomerulo-nephritis are rare among the domesticated animals, and that the acute form is also rare if one does not include under that head the frankly pyæmic cases in which the alterations in the glomeruli are demonstrably caused by the presence of streptococci or other bacteria. He has, however, encountered kidneys in which a considerable number of the Malpighian bodies contained crescents, and perhaps some of

* *Specielle pathologische Anatomie der Haustiere*, Band III, 1923, p. 294.

these cases might be classed as examples of the so-called catarrhal glomerulo-nephritis.

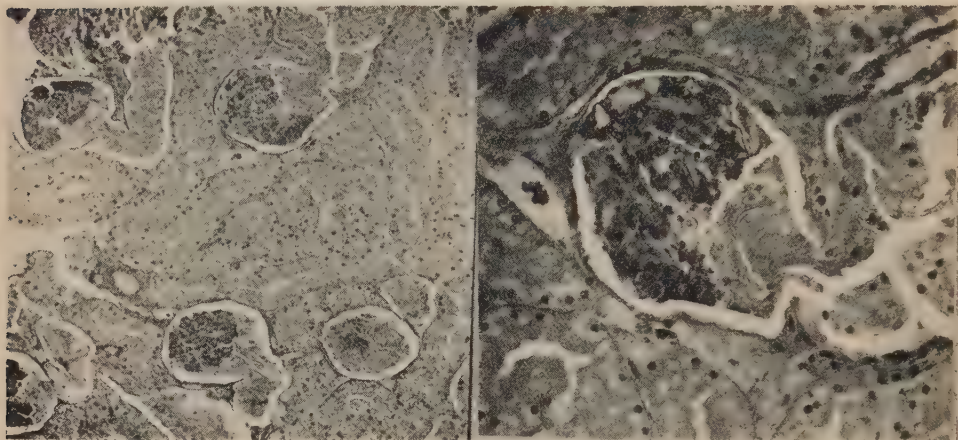


FIG. 10.

From same cat as Fig. 9. In the left half each Malpighian body shows a collection of epithelial cells sharing the capsular space with the glomerulus. In the right half one of these is seen under a higher magnification.

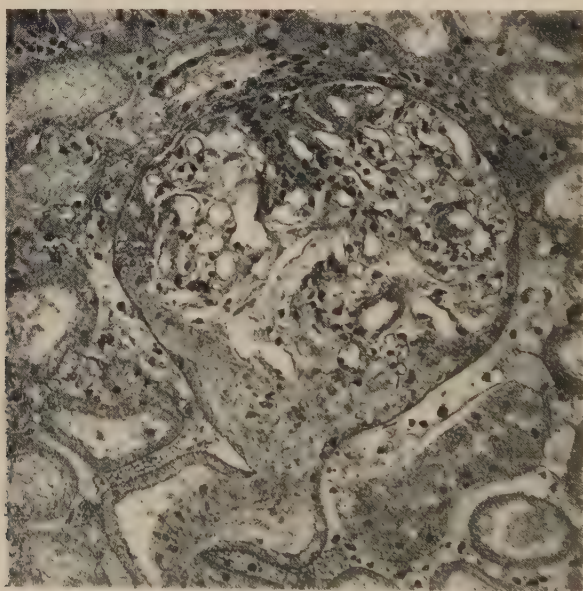


FIG. 11.

From the kidney of a cow. Shows degenerated epithelial cells apparently passing from Bowman's capsule into the tubule, the neck of which is dilated. The kidney contained red infarcts.

The first of these (in a cat) was described in this Journal in 1891 (Vol. IV., p. 238), and the note was accompanied by drawings showing the appearance of the lesions (Plate facing p. 241). The accompanying micro-photographs are from some of the more recent cases, and they may help to throw light on the manner in which the crescents are formed. It would appear doubtful whether the glomerular epithelium had any share in the formation of these bodies in the observed cases, although the view that they have that origin appears to be widely held. The figures suggest that the bodies are formed by desquamation of the capsular epithelium, and that the detached cells are carried in the exudate from the glomerulus towards the neck of the tubule, where, failing to escape, they accumulate between the glomerulus and the capsule, and must perforce exhibit a crescentic form on section. In none of the cases was there any evidence that either the capsular or the glomerular epithelium was in a condition of proliferation.

Unfortunately, in most of the cases no clinical history was obtained, but the cat from which Figs. 9 and 10 were taken died from streptococcus septicæmia. In both kidneys the cortex presented the diffuse whiteness of fatty degeneration, the medulla was congested, and the Malpighian bodies were not prominent. As Fig. 9 shows, the whole of the epithelial cells of the convoluted tubules were swollen and fatty. The great majority of the Malpighian bodies showed collections of epithelial cells, which appeared to be fatty in the same degree as those of the tubules. At no place was there any excess of leucocytes or other cells around the malpighian bodies or between the tubules.

In conclusion, the author would like to suggest that the relative frequency of the different recognised forms or types of nephritis in the domesticated animals requires further study, more particularly with regard to the occurrence of chronic forms in the horse, ox and pig, and what appear to be acute cases with enlargement of the kidneys in any species. It is very specially to be desired that those who may be able to record any such cases will endeavour to place the nature of the alterations beyond dispute by micro-photographs.

ANÆROBIC INFECTIONS IN LAMBS.

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INTRODUCTION.

In the sheep-breeding districts of the North of England the term "grass ill" is used to designate what is regarded as a specific disease of lambs. It is a seasonal disease occurring towards the end of May and also in June, or soon after the lambs begin to make grass part of their diet. Grass ill is well-known in the border counties, especially on the

higher-lying breeding farms, where lambing commences about the twelfth day of April. By the middle of May, in an ordinary season, the grass on these farms is growing more or less abundantly, the older lambs are eating greedily, and the appearance of the disease, coinciding as it does with the first flush of young grass, accounts for the name grass ill. The terms "sickness" and "grass sickness" are also used, the word "sickness" indicating a supposedly close relationship to Braxy as it occurs in hogs.*

References to grass ill in lambs occur among the earlier writings on Braxy published during the last century. In 1807, Duncan¹, who edited a series of essays on sheep diseases written by laymen, says "when about three weeks old and beginning to make grass a part of their food, especially if it be coming in great plenty, a straggling lamb or two will sometimes die of what is known as 'grass ill,' which, both from its symptoms, its speedy termination in death and appearances on dissection, seems to be a species of the disease described above" (i.e., Braxy).

McGowan² recognised grass ill as a disease of lambs, and it was considered by him to be identical with Braxy in hogs. He states that he saw ten cases of grass sickness, all of which occurred in the best and most thriving lambs. These lambs had apparently been perfectly healthy, and nothing wrong was noticed until they were found dead towards the end of May, when they were about one month old, or soon after they had begun to eat grass. The post-mortem appearances of these cases are stated by McGowan to have been identical, namely, "hæmorrhages all through the body, especially in the body muscles and on the surface of the heart, together with effusion into the pericardial sac." From all these cases McGowan states that the same organism was obtained, namely, *Bacillus bipolaris septicus ovium*. This organism he also believed to be the cause of Braxy in hogs. McEwen³ records that, out of seventy-four lambs which were put to graze on new pastures, thirteen died suddenly. From one of these cases *Vibrio septique* was isolated. Dalling⁴ and Pool⁵ mention grass ill when discussing lamb dysentery, but they do not state their views on its causation.

In Northumberland grass ill cannot be considered a serious disease. The mortality resulting from it varies, but it occurs every year. Some seasons it may account for 5 per cent. of the lamb crop, while in other years, for reasons not well understood, the loss is much lighter. Apart from the number of deaths, the disease is particularly vexatious to the breeder, inasmuch as many of the largest and most forward lambs become affected and are found dead.

It has been noted that the similarity of Braxy in hogs and grass ill in lambs is in many features most striking. Both diseases may, and usually do, occur on the same farms, and in both it is usually the best and most forward lambs which fall victims to the disease. Further, common to both diseases is the fact that affected animals are seldom seen alive, and the diagnosis is therefore generally made on post-mortem

* Young sheep of both sexes are known as hogs during their first winter of life.

appearances. On the other hand, recent investigations into lamb dysentery published by Dalling⁴ have established that a *Bacillus welchii* type of organism plays an important rôle in its causation. Our information showed that upon grass ill farms Braxy also occurs, but it is equally true that lamb dysentery is enzootic within this area, and we could not overlook the possibility of grass ill being an acute and rapidly fatal toxæmia produced by invasion with what Dalling has called the "L.D. Bacillus." Indeed, it has been suggested by several flockmasters that vaccination of ewes against dysentery in lambs with antigens prepared from a *Bacillus welchii* type of organism, isolated from naturally occurring cases of lamb dysentery, besides protecting against this disease also reduces the death-rate from grass ill. This possibility appeared to be at least feasible, because one of us (W. L. S.) has on several occasions seen the shepherd's diagnosis of grass ill disproved by post-mortem examination, and shown the disease to be in reality chronic lamb dysentery. Undoubtedly some old standing cases of lamb dysentery which cause death when the lamb is four weeks old are mistaken for cases of grass ill. At the same time we believe that for both diseases a distinct appearance exists, since chronic cases of dysentery are usually unthrifty lambs, but we have seen the diseases confused, and sometimes only a post-mortem examination can decide.

It was apparent that the etiology of this disease was imperfectly understood, and we thus availed ourselves of the opportunity to probe the matter further, not only on account of its academic interest, but also because the point has a certain practical bearing in view of the fact that both lamb dysentery and Braxy in hogs may now be regarded as preventable diseases. A preliminary enquiry was made during the 1928 lambing season, but most of the work herein detailed was carried out during the 1929 season.

PRELIMINARY ENQUIRY. SEASON 1928.

During the 1928 lambing season we examined three cases of grass ill, one case being obtained alive and the other two dead. These cases were seen in the field, and no bacteriological work was done, the examination being limited to a careful study of the pathological appearances of the various organs. Information was also gathered relating to the occurrence and history of the disease, and, although this information was added to in 1929, it will be convenient here to give an account of the clinical aspect of grass ill.

Symptoms.—Altogether three living cases of grass ill were obtained, one in 1928, and two in 1929, and we had thus an opportunity of observing the symptoms shown. They may be described as follows. An affected lamb has a very slow gait. While the other members of the flock keep well ahead of the shepherd on their way downhill in the morning or uphill at night, an affected lamb lags behind and attracts the shepherd's attention. If such a lamb, obtained in the early stages, be placed under observation, it will be seen to exhibit symptoms of great uneasiness. It moves restlessly about the sheep-pen with the back slightly arched and the mouth open to assist respiration, which is

short and rapid. The lamb does not feed, and when the udder of the ewe is examined it is seen that the young animal has not sucked for perhaps several hours. The symptoms become aggravated, but very often the lamb does not lie down until near the point of death. It remains standing with the eyes half closed and a watery discharge trickles from the inner canthus. There is a rise in temperature to as high as 107° F., which we regard as abnormally high for lambs, especially in cold weather. In general the lamb has a listless and depressed appearance, shows evidence of great abdominal pain with elevation of temperature, as though it had suddenly been stricken with a very acute and painful indigestion. One case was observed for two hours, and during that time the lamb made no attempt to pass urine or fæces. We were informed by shepherds that the tails and buttocks of lambs dead from grass ill are often soiled with fæces, and that diarrhœic stools are sometimes found in the vicinity of the lamb, suggesting that copious evacuation of the bowel had taken place shortly before death.

As has already been stated, it is rarely that an affected case is seen alive, and the shepherd's diagnosis of grass ill is based not upon the symptoms shown but usually upon the external appearances after death. With these appearances the hill shepherd is familiar, and when a lamb about four weeks old is found dead and stretched, the abdomen distended with gas, and showing diarrhœa and a nasal discharge, death is attributed to grass ill. After death a strong putrefactive smell is said to occur.

In the district where our work was done grass ill, louping ill, and lamb dysentery are common, and a real difficulty exists in distinguishing these diseases while affected animals are still alive. The disease known as louping ill in lambs is particularly liable to be confused with grass ill, and we have been able to show that sometimes they may only be distinguished post-mortem.

Post-mortem Appearance.—The organs from the three cases obtained in the spring of 1928 were carefully examined. Sections were cut for histological work, but a full discussion of these changes will be deferred to the protocols.

INVESTIGATIONS IN SEASON 1929.

It appeared to us essential to have facilities for carrying out laboratory work on farms where grass ill occurs. The disease is of such a rapidly fatal character that our only chance of obtaining living affected cases seemed to be to concentrate our efforts on one or two sheep farms, where the whole-hearted co-operation of owners and shepherds was assured. We were fortunate in receiving this support, and preparations were commenced early in 1929. A suitably constructed stone building, conveniently situated near three farms where the disease was said to be enzootic, was kindly put at our disposal by Sir Claud Morrison Bell, of High Green, Northumberland, and this building was converted into a laboratory. It consisted of a well-lighted outhouse, free from dust, with good bench room and shelving, and an abundant water supply. A small sheep-pen was erected near the laboratory in order that living

cases might be observed until near the point of death. The laboratory was equipped by making use of the existing material in our main laboratories at the University. In the absence of supplies of gas and electricity, paraffin and petrol stoves and lamps supplied us with a source of light and heat for sterilisation and incubation purposes. A post-mortem table was available. Freshly prepared tubed media were brought from the central laboratory.

It may here be said that the difficulty in obtaining living cases of grass ill, in spite of our arrangements, proved a very real one, and we desire to record our appreciation of the help we received from the farmers and shepherds who supplied us with the cases of the disease which form the basis of this article.

Field laboratory technique.—Arrangements were made for complete bacteriological and pathological examination of all affected cases. For bacteriological work the following types of media were used for aerobic cultivation :

- (1) Agar and glucose agar slopes.
- (2) Serum agar and glucose serum agar slopes.
- (3) Ordinary broth and glucose broth.
- (4) Serum broth and glucose serum broth.
- (5) Tryptamine blood extract agar.
- (6) Solid serum slopes.

The basis of all media with exception of the last was a casein digest.

Under field laboratory conditions it was not found possible to employ full anærobic equipment, and use was made of only two liquid types of media that had proved satisfactory in our hands for the growth of all pathogenic anærobcs known to us. These were :—

(1) Chopped meat broth. This medium consists of an extract prepared from horse flesh with peptone (Witte's) and sodium chloride added. A little of the chopped meat is added to each tube. No surface layer of liquid paraffin is used.

(2) Liver extract broth. This medium consists of an infusion of sheep's liver with peptone and sodium chloride added. A surface layer of liquid paraffin is used.

The anærobic media were boiled for ten minutes and rapidly cooled immediately before use.

All media were adjusted to a pH of 7.6–7.8.

On arrival at the laboratory cases were dealt with as follows :—

If alive, the lamb was placed under observation and killed by throat bleeding when near the point of death. The same procedure was now followed for all cases brought to the laboratory. Lambs were skinned by the shepherd's method, taken within the laboratory, and securely fixed on the post-mortem table, ventral surface upwards. Preparation of media and instruments was now completed, and the bacteriological examination commenced.

On the right side of the abdomen a broad band was seared with the actual cautery. A small incision was carefully made here with a sterile scalpel, and the cut surface held by sterile forceps while some of the peritoneal fluid was withdrawn in a Pasteur pipette. Inoculations were made into aerobic and anærobic media. Smears of peritoneal fluid were made for microscopical examination later. The liver was next exposed and, as it lay untouched, its surface was seared with the iron. A liver emulsion was secured by means of a pipette plunged into the parenchyma, and small portions transferred to tubes of media. Liver impression smears were made from other lobes. Portions

of the inflamed bowel wall were now processed. Held securely by two pairs of artery forceps, a small portion of the inflamed area was swabbed with sterile cotton wool rinsed in crude lysol. A new sterile scalpel was used to incise and reflect the serous coat, and inoculations and smears were made with a platinum loop from the blood-stained exudate. Emulsions were obtained from the mesenteric and stomach glands by piercing their seared surfaces with pipettes. The thorax was now seared through on the right side and inoculums of pleural fluid aspirated. After the sternum was removed with bone forceps, pericardial fluid was taken in a pipette passed into the sac, which was seared near its apex. The heart was now exposed, seared over the left ventricle, and blood withdrawn for cultural work. Inoculations were made from kidney, spleen, and suprarenal gland. The capsule of one kidney was seared through and portions of the cortex removed, using sterile scalpels and forceps. Material from spleen and suprarenal gland was obtained by passing a pipette through the seared surface.

All tubes of media were incubated at 37° C., and the period of primary incubation varied from 6 to 16 hours according to the time the case was received. When a lamb arrived in the morning microscopic examination of cultures was made as soon as macroscopic signs of growth appeared in any tube. With all positive anærobic tubes sub-cultures were made immediately in anærobic and ærobic media and incubated 18-24 hours. Mother cultures were re-incubated until 24 hours old. The culture tubes from cases received in the evening did not receive attention for 12 to 16 hours. In all cases inoculations were not regarded as having yielded negative results until the cultures had been incubated 72 hours at 37° C. and subcultures had proved negative after 48 hours incubation.

As soon as the bacteriological examination was completed the organs were examined for pathological changes and material preserved for histological work.

RECORDS OF POST-MORTEM EXAMINATIONS.

Case L1.—Received May 9th, 1929. Time, 6 p.m. A well-developed Blackface male lamb, three weeks old. Found dying by shepherd, and immediately brought to field laboratory. The shepherd diagnosed the case as typical grass ill, and stated that it had been seized with illness after 3 p.m., as he had observed the lamb then and it appeared perfectly healthy. When the dam was caught her udder was found to contain no excess of milk, which seemed to confirm the shepherd's statement. This lamb was observed for fifteen minutes, and was killed when comatose.

Post-mortem.—There was slight hæmorrhage into subcutaneous tissues and congestion of larger vessels. Abdomen was distended with gas; no putrefactive smell. About 20 ounces of blood-stained peritoneal fluid was present, and fluid was also present in pleural cavity and pericardial sac. Liver dark, engorged with blood, and showed blackish areas. Kidneys were congested, markedly soft, and showed through the capsule with a dull bluish, almost metallic, lustre. There were numerous petechial hæmorrhages present on cortex. Lungs congested; copious blood-stained frothy discharge exuded into bronchi and trachea. This was also evident at the nostrils. No lesion detected in fourth stomach. Small intestines congested throughout, but with no particular intensity except in first foot of duodenum. The true stomach contained partly digested grass, curdled milk, and black particles derived from the burnt heather. No hair or wool ball. All lymphatic glands throughout the body were congested, especially those in the mesentery, which exuded a quantity of turbid fluid when cut. The coronary vessels distended with blood. Endocardium showed a cluster of small hæmorrhages in the neighbourhood

of the columnæ carneæ. The other organs appeared normal. Blocks of tissue for histological work were taken from kidney, liver, lung, heart, glands, and duodenum.

Apparently pure growths of an actively dividing, slender, Gram-positive bacillus were obtained from peritoneal fluid, liver, small intestine, mesenteric gland, and kidney. Inoculations of heart blood in all media gave negative results. Small intestine, pleural fluid, and suprarenal gland gave aerobic growths in glucose serum agar. These organisms were later identified as being indistinguishable from *Bacillus coli communis*. Chopped meat broth cultures retained for identification were: peritoneal fluid L1 (6), liver L1 (4), small intestine L1 (5), mesenteric gland L1 (3), kidney L1 (2).

Case L2.—Received 9th May, 1929. Time, 7.30 p.m. A Blackface lamb, four weeks old. This lamb was found ill by shepherd, who was uncertain whether it was a case of "wool ball" or grass ill. It showed great excitability, jerked its head and stamped with its forefeet, as though the brain was affected. Later diagnosed as not grass ill, but the lamb was killed, and usual examination carried out.

Post-mortem.—Internal organs appeared perfectly healthy. There was no "wool ball," and no evidence of grass ill or lamb dysentery. This lamb may have been affected with acute louping ill, although we failed to demonstrate the presence of chromatin bodies in the leucocytes, as described by Stockman (6).

A complete bacteriological examination of all body fluids and organs was carried out. In no single case was a bacterial growth obtained.

Case L3.—Received 10th May, 1929. Time, 9 a.m. Single Blackface lamb, two weeks old. Found alive, but unable to stand. There was slight diarrhœa, and the case was diagnosed as lamb dysentery.

Post-mortem.—Peritoneal fluid plentiful, turbid, and contained flocculi. About ten feet of the jejunum and ileum were acutely inflamed, and the walls were studded with the small bubbles of gas typical of acute lamb dysentery infection. Mucous membrane of bowel necrotic. Bowel contents dark, and in parts almost entirely replaced by blood. Diagnosed as lamb dysentery.

Inoculation of liver in chopped meat broth gave an apparently pure growth of a short square-ended bacillus; aerobic subcultures proved negative. Mixed growths of Gram-positive and negative bacilli were obtained from peritoneal fluid and mesenteric glands. No growth was obtained from heart blood, pericardial fluid, kidney, spleen, and suprarenal gland. One subculture from liver growth (L3 [2]) was retained for identification.

Case L4.—Received 14th May, 1929. Time, 7.30 p.m. Blackface lamb, three weeks old. This lamb was not observed ill, and was discovered dead by the shepherd at 6 p.m. Symptoms of diarrhœa present. Case diagnosed by shepherd as grass ill.

Post-mortem.—Abdomen tense, no frothing at nostrils. Subcutaneous vessels were injected with blood. There was a large amount of blood-stained peritoneal fluid, and similar fluid was present in thorax. The small intestines were markedly inflamed, especially in region of duodenum. Mesenteric glands also inflamed. The kidneys were soft, and showed many hæmorrhages. Endocardium also showed many points of hæmorrhage. Liver in state of cloudy swelling. No fourth stomach lesion. All the other organs appeared normal. Block of tissue taken from the various organs for histological work.

Cultures from peritoneal fluid, liver, mesenteric gland, and heart blood gave mixed growths of Gram-positive and negative bacilli and Gram-positive cocci in anærobic media. One chopped meat broth subculture from liver (L4 [1]) was retained for identification.

Case L5.—Received 15th May, 1929. Time, 9 a.m. This lamb, a Black-face male, two weeks old, was found dead on the hill at 6 a.m. It had not been observed ill. Case diagnosed by shepherd as lamb dysentery.

Post-mortem.—Post-mortem showed that in all probability death was due to rupture of the wall of the fourth stomach. Both peritoneal and pleural cavities contained a quantity of liquid milky ingesta from the stomach. The whole of the small intestine was punctuated with small healing lamb dysentery ulcers with yellow centres. All other organs appeared normal.

An apparently pure culture in anærobic media of a short square-ended bacillus was obtained from the liver. Aerobic subcultures proved negative. Peritoneal fluid yielded a mixed growth in ærobic media. One liver subculture (L5 [1]) was retained for identification.

Case L6.—Received 15th May, 1929. Time, 6 p.m. Lamb 6 was known to be three hours dead, and was only examined in want of better material. It was a strong Blackface male, four weeks old, and it was stated to be a typical case of grass ill. The shepherd found this lamb stretched out, dead and cold, at 3 p.m.; it had not been noticed ill. There was evidence of diarrhœa, and the abdomen was distended with gas.

Post-mortem.—Peritoneal, pleural, and pericardial fluids present. Liver and kidney showed marked toxic change. The intestines were not inflamed, but the fourth stomach showed patchy inflammation in discrete areas about the size of a pea. Five such areas were noted. This lamb showed more clearly than the others the post-mortem appearances said to be associated with typical Braxy (Gaiger).

Peritoneal fluid, liver, mesenteric gland, and heart blood gave mixed growths in ærobic media of a slender Gram-positive bacillus, together with Gram-negative organisms. One heart blood subculture (L6 [2]) was retained for identification.

Case L7.—Received 16th May, 1929. Time, 6 p.m. Single Blackface male lamb, three or four weeks old. This lamb was found by the shepherd at 5 p.m., suffering from clinical grass ill. It stood apart from its mother, and was disinclined to move, the back was arched, and breathing was accelerated. It was immediately sent to the laboratory, where it was placed under observation. Soon after it was placed in the sheep pen its temperature was taken per rectum, and was found to be 105° F. There was no change in the lamb's condition forty minutes later, and it was decided to kill it and commence the examination.

Post-mortem.—The abdomen was tense and full, but not over-distended. Slight congestion of the subcutaneous vessels. Blood-stained peritoneal, pleural, and pericardial fluid present in considerable amount. There was a little inflammation of the small intestines, but not so marked as in Case L4. Mesenteric glands congested. Kidney showed same appearances as Case L1. Liver enlarged, pale, showed cloudy swelling, and the edges were rounded. Spleen enlarged, and darker than usual. Fourth stomach contained a mixture of grass, curdled milk, and black particles. The blood vessels on stomach wall congested, but no areas of inflammation could be detected. Towards the end of small intestine there were two old-standing and almost healed lamb dysentery ulcers, surrounded by thickened areas. Lungs congested. The endocardium showed several hæmorrhages, but were not so marked as in Case L1. All the other organs appeared normal. Blocks of tissue for histological work were taken from liver, spleen, kidney, and inflamed small intestine.

Chopped meat broth cultures from peritoneal fluid, liver, small intestine, mesenteric gland, heart blood, kidney, and spleen were apparently pure growths of a slender Gram-positive bacillus. All ærobic sub-cultures gave negative results. No growth was obtained from pericardial fluid and suprarenal gland.

The cultures retained were from peritoneal fluid L7 (4), liver L7 (5), small intestine L7 (1), mesenteric gland L7 (3), heart blood L7 (2), kidney L7 (7) and spleen L7 (8).

Case L8.—Received 18th May, 1929. Time, 8 a.m. A Blackface male lamb, four weeks old, was found dead by shepherd during the morning round. Diagnosed as a typical case of grass ill.

Post-mortem.—There was a large amount of blood-stained peritoneal fluid, also a quantity of pleural and pericardial fluids. Areas of intense peritonitis, especially over small intestines. About two yards of jejunum showed the intense inflammation and diffuse ulceration which is associated with acute dysentery. The mesenteric gland superior to this part was inflamed. Lungs congested, also coronary blood vessels. No hæmorrhages in endocardium. Spleen inflamed. Kidneys soft and pulpy, but not markedly inflamed. Liver toxic. All other organs appeared normal. Blocks of tissue from the various organs were taken for histological work.

Mixed growths in anærobic media were obtained from peritoneal fluid, liver, mesenteric gland, heart blood, and kidney. Gram-positive and negative bacilli were abundant in primary culture. One subculture ex liver (L8 [1]) was retained.

Case L9.—Received 19th May, 1929. Time, 10.30 a.m. This was a strong Blackface male lamb of four weeks, found dead on morning round. It was diagnosed as typical grass ill by the shepherd.

Post-mortem.—Post-mortem examination of this lamb revealed the same appearances as have been described for Case L1. In addition, the spleen was enlarged and mottled with small purplish areas. There was a quantity of frothy exudate in trachea and bronchi; lungs were congested. Blocks of tissue were taken from the various organs.

All body fluids and organs gave mixed growths of Gram-positive and negative bacilli and Gram-positive micrococci.

One subculture in anærobic media from liver (L9 [4]) was retained.

Case L10.—Received 20th May, 1929. Time, 7.30 p.m. This lamb had been dead for several hours when examined. It was a large Blackface male lamb. Stated to have died from grass ill.

Post-mortem.—Abdomen distended with gas. The lamb may have been affected with grass ill, but the wall of the fourth stomach was found ruptured and contents were scattered over peritoneal cavity. Pleural and pericardial fluids present. There was a little discoloration of mesenteric glands. No obvious change on mucous membrane of either stomach or intestines. The kidneys were soft and pulpy. No hæmorrhages. Liver enlarged and pale. Lungs congested. Heart appeared normal.

Mesenteric gland and heart blood yielded mixed growths of Gram-positive and negative bacilli in anærobic media incubated 24 hours at a little above room temperature. One culture from the mesenteric gland (L10 [3]) was retained.

Case G.—Received 12th May, 1929. Time, 7 p.m. This case was a gimmer (two-year-old ewe), which the shepherd said had died from Braxy. It was newly dead when it arrived at the laboratory, and it was decided to carry out a complete bacteriological examination.

Post-mortem.—The abdomen was enormously distended with gas and blood-stained peritoneal fluid, and the whole carcass had a disagreeable odour of putrefaction. The post-mortem changes were confined to the abdominal cavity. The fourth stomach, *in situ*, showed no naked-eye lesion, and none could be detected when the organ was opened and washed. The small intestines were intensely inflamed throughout. Liver, kidneys, and suprarenal glands showed evidence of toxic change. The other organs appeared normal.

On anærobic media apparently pure cultures of a slender, round-ended Gram-positive bacillus were obtained from peritoneal fluid, liver, mesenteric gland, and heart blood. All ærobic primary cultures and ærobic subcultures from anærobic tubes gave no growth.

Cultures retained for identification were from peritoneal fluid G (4), liver G (2), mesenteric gland G (1), and heart blood G (3).

PATHOLOGICAL HISTOLOGY OF GRASS ILL.

Of the eleven cases dealt with in the protocols, only six (Cases L1, L4, L6, L7, L8, and L9) were regarded by the shepherds as certain cases of grass ill, and of this number only Cases L1 and L7 were brought to the laboratory alive. Accordingly, in describing the pathological histology of grass ill, we will eliminate the risk of discussing changes which may have occurred after death by limiting ourselves to the use of tissues and material obtained from the two living cases. In passing it may be stated that sections were examined microscopically from the four cases which were received after death had occurred, and in our opinion the pathological changes observed were substantially those which are now described.

Fluids.—Smears made from the peritoneal fluids of L1 and L7 were stained with Gram's and Loeffler's alkaline methylene blue stains. They showed the presence of a long, slender, round-ended, weakly Gram-positive bacillus occurring singly and in pairs. They were not plentiful, and many fields did not show any. No sporing forms were detected. The fluid from cut mesenteric glands also showed these bacilli. They were not demonstrated in the other fluids.

Kidney.—Cortex. L1. This area shows numerous large hæmorrhages following no characteristic distribution, but they are more marked towards the free border. In the neighbourhood of the hæmorrhages there is necrosis of convoluted tubules, with complete loss of structural outline and karyolysis of the nuclear chromatin. A few red blood corpuscles are seen in Bowman's capsule, due perhaps to glomerular hæmorrhage. There is progressive degeneration of the glomerular capsule and convoluted tubules, and in some of the tubules red blood corpuscles and cellular débris are noted.

Medulla L1. The capillaries and venules are dilated with blood and small hæmorrhages occur throughout the medulla. The degeneration of the kidney tubules varies from typical cloudy swelling to total destruction and desquamation of the lining cells. Especially in the convoluted tubules the cells are seen detached from the stroma of the wall and lying free in the lumen. The structural outline of the collecting tubule is well preserved. Sections stained with Loeffler's methylene blue did not reveal any bacteria. The lesion is compatible with an acute toxæmia or septicæmia. The scarcity of cellular exudate indicates an acute infection followed by rapid death.

Case L7. The appearance of the kidney from L7 is similar to the above described, and warrants no special description.

Liver.—Case L1. The sinusoids are congested and the central lobular vein is markedly congested. Around the central vein there is a

little hæmorrhage and some necrosis of parenchymatous cells. In general there is cloudy swelling of liver cells, and the cytoplasm is granular, this being most marked near centre of lobule, but the condition is not intense.

Case L7. The liver from this case shows intense cloudy swelling, hæmorrhages in parts, and regular zonal necrosis, particularly near the centre of lobule. In some of the venules there appear to be micro-organisms.

Lung.—Case L1. The capillaries are dilated and engorged with blood. A few bronchi contain a little serous exudate and show slight catarrhal change. Some alveoli also show catarrhal change and a few red blood corpuscles. The condition is one of acute congestion with œdema, and there is no pneumonic consolidation.

Case L7. The lungs are acutely congested. No consolidation. There is a catarrhal change in the bronchi. The alveoli contain red blood corpuscles and serous exudate so as to constitute a very early form of broncho-pneumonia.

Mesenteric lymphatic glands.—Case L1. There is a lymphoid hyperplasia. No definite reticulum-hyperplasia. Only a few polymorphonuclear leucocytes can be seen in the lymph sinuses, the majority of the leucocytes being of the mononuclear variety. There is no hæmorrhage, and the condition is one of acute lymphadenitis.

Case L7. Gland shows condition of acute lymphadenitis with polymorphonuclear and mononuclear leucocytes, which are actively phagocytic. These cells appear to contain a brownish pigment which may be derived from the blood.

The other lymph glands show a similar condition.

Small Intestine.—Duodenum. Case L1. The mucous membrane shows an acute catarrhal inflammatory process with infarcts, definite destruction and necrosis of the lining epithelium, and fibrinous exudate. There are many little hæmorrhages within the mucous and sub-mucous coats. Sections stained with Loeffler's methylene blue show an enormous number of long slender bacilli in chains invading the tissue.

Case L7. Duodenum. Shows an acute inflammation of mucous and sub-mucous coats, with a deposit of fibrin and presence of many leucocytes. Long filaments of slender bacteria are seen invading the mucous and sub-mucous coats.

Spleen. Case L7. The spleen from L1 was not examined. Sections from L7 show this organ to be acutely congested. Malpighian bodies unaffected. There is some hæmorrhage and much blood pigment lying free, and there are numerous filaments of long slender bacteria throughout the splenic pulp. There are also a number of polymorphonuclear leucocytes in the spleen pulp.

EXPERIMENTAL BACTERIOLOGY.

From the preliminary isolation work carried out at the field laboratory and from the pathological information collected there, we had evidence that anærobic infections were probably responsible for the death of most of the cases examined. The work which followed at the

central laboratory was therefore directed to (a) the determination of purity of the cultures of anærobes isolated; (b) an endeavour to set up the disease grass ill in normal lambs, using strains isolated from a typical living case of the disease; and (c) the classification of the organisms isolated according to their morphological, cultural, and immunological characteristics.

(a) *Determination of purity of cultures.*—This was carried out by a system of alternate rapid subculturing in liquid media and plating on glucose casein digest agar, to which had been added sheep's serum and hæmolyzed red blood cells. McIntosh and Fildes jars were used for obtaining growths on solid media. Between each plating a constant check on anærobic technique was made by subculturing on aerobic media. It was found that in all cases mentioned under field protocols where there was indication that we were dealing with pure cultures the colonies on plates appeared uniform. In other cases single colonies of the various types identified were sub-planted on aerobic and anærobic media. Aerobic growths were obtained in a few cases, but the type of organism was not constant, being principally coliform and coccidial forms. All aerobes were discarded.

For subsequent work a single culture was selected from each case after plating six times. These cultures were L1 (6), L3 (2), L4 (1), L5 (1), L6 (2), L7 (5), L8 (1), L9 (4), L10 (3), and G (1). The plating of these cultures was carried out a further seven times, and they were then passaged through guinea-pigs. Cultures from the livers of guinea-pigs *in extremis* were obtained and plated. This process was repeated, and we now assumed that we were dealing with pure cultures. Two preliminary "sugar" fermentation tests in peptone water were now carried out. With the exception of cultures L4 (1) and L8 (1), with which we were unable to infect guinea-pigs, all other cultures proved highly pathogenic. These two cultures were strict anærobes, and their classification was left over till a later date.

A study of the guinea-pig reactions and "sugar fermentation" tests gave us the preliminary key to the identification of cultures, and showed that they might be grouped as follows:—

L1 (6)	}	Reactions similar to known strain of <i>V. septique</i> . (National Type Culture.)
L6 (2)		
L7 (5)		
L9 (4)		
G1		

L3 (2)	}	Reactions similar to known strain of Dalling's " <i>L.D.</i> <i>Bacillus</i> ." (W.P.R.L.)
L5 (1)		

L10 (3)	{	Reaction similar to known strain of <i>B. welchii</i> . (National Type Culture.)
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(b) *Artificial Infection of Young Lambs.*—Only two normal lambs were available. They were six weeks old at the time of experiment, and had been kept at the laboratory for four weeks.

Inoculation Experiment.—Lamb No. 1. 4th June, 1929. 12.45 a.m. 2.5 c.c. eight-hour meat broth culture of L7 (5) inoculated subcutaneously on inner aspect of right thigh. 9 a.m., lamb found gasping and unable to stand. It was killed at 9.30 a.m., and post-mortem examination conducted at once.

Pathological.—There was a well-marked, gelatinous, emphysematous, inflammatory œdema in region of groin, where the muscles were extensively infiltrated. Blood-stained peritoneal and clear pericardial fluids were present. The endocardium of the left ventricle showed hæmorrhages near the columnæ carneæ. Small intestines, mesenteric glands, and kidneys intensively inflamed. The liver enlarged and dark in colour. All other organs appeared normal.

Bacteriological.—Pure cultures of the anærobe were obtained from peritoneal fluid, liver, heart-blood, mesenteric gland, kidney, spleen, and suprarenal gland. These cultures were subsequently tested for purity by plating. Heart blood culture (C [1]) was retained for identification. No growth was obtained on aerobic media.

Feeding Experiment.—Lamb No. 2. This lamb received water but no food for 48 hours prior to commencement of experiment.

9th June, 1929. 11.30 a.m. 200 c.c. of sodium bicarbonate solution was administered by stomach tube, and immediately followed by 25 c.c. of a 24-hour meat broth culture of L7 (5). Another 25 c.c. of the same culture was mixed with cut grass and fed to lamb.

10th June, 1929. Lamb dull, but still eating.

11th June, 1929. Lamb dull.

12th June, 1929. Lamb appeared normal.

13th June, 1929. 11 a.m. Lamb fed 25 c.c. of a mixture of C (1) and L7 (5) by stomach tube. Twenty-hour meat broth sub-cultures were used.

14th June, 1929. Lamb found dead, 9 a.m. Post-mortem conducted at once.

Pathological.—Subcutaneous hæmorrhages present in thoracic region. There was a quantity of blood-stained peritoneal and pleural fluids and about 5 c.c. of turbid pericardial fluid. The coronary blood vessels were distended, and hæmorrhages were present on the endocardium, especially in the left ventricle. Lungs were congested. The blood vessels of fourth stomach engorged with blood, and the mucous membrane of this organ showed numerous small hæmorrhages. Last four feet of small intestine were inflamed, and all lymphatic glands in abdomen highly congested. The kidney showed cortical hæmorrhages, and the liver appeared dark in colour. Blocks of tissue from the various organs were taken for histological work, and microscopic examination showed the same anatomical changes as described for the naturally occurring cases of grass ill.

Bacteriological.—Microscopic examination of the peritoneal fluid and liver impression smears showed the presence of long chains of slender round-ended bacilli; sporing forms were also observed. Pure cultures of the anærobe were obtained from heart-blood and liver. Cultures from pleural fluid, kidney, and mesenteric glands showed a

mixed growth of the anærobe and a Gram-negative organism. These mixed cultures were discarded. Retained cultures were tested for purity by plating, and that from liver (C [3]) used for identification.

(c) *Morphological, Cultural, and Immunological Identification.*—Cultures L1 (6), L6 (2), L7 (5), L9 (4), G (1), C (1) and C (3), grown on the various artificial media, presented naked-eye and microscopic appearances indistinguishable from those of a known strain of *V. septique* obtained from the National Collection of Type Cultures. Cultures L3 (2), L5 (1), and L10 (3) were morphologically indistinguishable from a National Type Culture strain of *B. welchii*.

Cultural.—The cultures which corresponded morphologically with the known strain of *V. septique* gave "sugar fermentations" which were constant in both the preliminary peptone water tests and final tests in peptone water and Hiss' serum water. The growth characteristics of cultures L3 (2) and L5 (1) on solid serum, alkaline egg, and glycerine were identical with those produced by a known strain of the "*L.D. Bacillus*" obtained from the Wellcome Physiological Laboratories. In previous work with strains of the "*L.D. Bacillus*" isolated by us, the "acrolein test" was found unsatisfactory, and it was not incorporated as a standard test in this work. Culture L10 (3) gave growth reactions on the differentiating media identical with the known strain of *B. welchii*.

It may be noted here that our known strains of the "*L.D. Bacillus*" and cultures L3 (2) and L5 (1) proved constant in their fermentation of raffinose, and quite as constant in their inability to ferment sorbite, and *vice versa* for the known strains of *B. welchii* and culture L10 (3). These ferment sorbite and not raffinose. As this had not previously been recorded, we decided to test the matter further. We have investigated these reactions, using five known strains of the "*L.D. Bacillus*" and three known strains of *B. welchii*, and this preliminary work confirms our original observation. This may prove to be an additional point of differentiation between these organisms.

Immunological.—The preparation of toxin in small quantities of approximately 25 c.c. presented some difficulty. Finally horse flesh medium with the addition of .3 per cent. glucose was inoculated with a portion of the liver of a guinea-pig dying from infection with the particular strain under investigation, together with, approximately 2 c.c. of a 24-hour culture in glucose meat broth. The period of incubation for toxin production varied with the strain from 18-36 hours. The cultures were filtered through a Seitz-Werke filter, using the Germicide E.K. filter pad. Filtrates were used immediately for intravenous inoculation in mice, and the minimum lethal dose estimated. Titration experiments with known high-titred antitoxins of *V. septique*, the "*L.D. Bacillus*," and *B. welchii* were set up, using 1 to 10 M.L.D.'s of the toxin, with .01 to .3 c.c. of the various antitoxins. Deaths were recorded to 24 hours.

Cultural experiments on guinea-pigs were carried out as follows:—One actively growing culture of each strain was subcultured in 30 c.c. of meat broth, and incubated 24 hours at 37° C. This culture acted

as stock, and all subcultures were made in glucose meat broth from this tube. The M.L.D. of cultures was estimated by the intramuscular inoculation of guinea-pigs, and deaths were recorded until the fourth day.

Antitoxin experiments using high titred sera prepared from known strains of *V. septique*, "*L.D. Bacillus*," and *B. welchii*, were set up as follows :—for each culture to be identified a series of three guinea-pigs was used, animals receiving 1 c.c. of the antitoxin of *V. septique*, "*L.D. Bacillus*," and *B. welchii* respectively. After 24 hours each guinea-pig received 2 M.L.D.'s. of the culture under test, and deaths were recorded until the fourth day.

TABLE.
ANTITOXIN EXPERIMENTS IN GUINEA-PIGS.

Culture.	M.L.D.		<i>V. Septique</i> Antitoxin, 1 c.c.	<i>L.D. Bacillus</i> Antitoxin, 1 c.c.	<i>B. Welchii</i> Antitoxin, 1 c.c.
L1 (6)	·01 c.c.	Left Thigh. Intra-muscularly, Inoculated 2 M.L.D.'s.	Live 4 days ...	F.D. 48 hours	F.D. 48 hours.
L6 (2)	·025 c.c.		Live 4 days ...	F.D. 36 hours	F.D. 36 hours.
L7 (5)	·01 c.c.		Live 4 days ...	F.D. 22 hours	F.O. 4 days.
L9 (4)	·05 c.c.		Live 4 days ...	F.D. 36 hours	F.D. 22 hours.
L3 (2)	·01 c.c.		F.D. 32 hours	Live 4 days ...	F.D. 48 hours.
L5 (1)	·005 c.c.		F.D. 36 hours	Live 4 days ...	F.D. 36 hours.
C (1)...	·01 c.c.		Live 4 days ...	F.D. 36 hours	F.D. 3½ days.
C (3)...	·01 c.c.		Live 4 days ...	F.D. 48 hours	F.D. 60 hours.
G (1)...	·01 c.c.		Live 4 days ...	F.D. 48 hours	F.D. 18 hours.
L10 (3)	·05 c.c.		F.D. 36 hours	Live 4 days ...	Live 4 days.
<i>L.D. Bacillus</i> (W.P.R.L.)	·005 c.c.		F.O. 48 hours	Live 4 days ...	F.O. 36 hours.
<i>V. Septique</i> (M.C.T.C.)	·01 c.c.		Live 4 days ...	F.D. 36 hours	F.D. 36 hours.

These animal experiments confirmed the earlier cultural work, and showed (a) that strains L1 (6), L6 (2), L7 (5), L9 (4), G (1), C (1), and C (3) were immunologically identical with a known strain of *V. septique* (N.T.C.) ; (b) that L3 (2) and L5 (1) gave the same reactions as the known strain of Dalling's "*L.D. Bacillus*" (W.P.R.L.) ; and (c) that the immunological characteristics of the known strains of *B. welchii* were indistinguishable from those of strain L10 (3).

Finally, all cultures were plated and single colonies sub-planted in meat broth. Twenty-four hour subcultures were inoculated in

peptone water, and Hiss's serum water "sugars" and the final fermentation reactions of each strain were recorded four times with the following results :—

Strains V. septique, L1 (6), L6 (2), L7 (5), L9 (4), C (1), C (3), and G (1) ferment lactose, glucose, maltose, galactose, lævulose, salicin, and mannite.

Strains "L.D. Bacillus" L3 (2) and L5 (1) ferment lactose, glucose, saccharose, maltose, galactose, lævulose, dextrin, mannite, and raffinose.

Strains B. welchii and L10 (3) ferment lactose, glucose, saccharose, glycerine, maltose, galactose, lævulose, dextrin, mannite and sorbite.

DISCUSSION.

It has been noted that several of the older writers on sheep diseases believed that Braxy and grass ill were identical diseases, and this belief is still shared by many farmers at the present time. McGowan likewise was of this opinion, but he stated that grass sickness was caused by an organism which he termed *Bacillus bipolaris septicus ovium*. We think it remarkable that the report of the Braxy Committee⁷ contains no reference to grass ill, since many enquiries were conducted by this Committee in Northumberland. Knowledge regarding anærobic infections of animals, however, has been greatly extended during the past ten years, and we now know that Braxy is caused by infection with the V. septique.

Grass ill is sometimes confused with deaths in lambs from intestinal obstruction due to "hair" or "wool" ball. Again, in some districts there is a belief that preventive treatment directed against lamb dysentery is also effective in reducing the death-rate from grass ill, thereby implying that dysentery and grass ill are similar if not identical diseases. Louping ill is common on many sheep farms in the border district, and acute cases among lambs are not easy to distinguish from grass ill. Our investigation was an endeavour to elucidate the etiology of grass ill.

We regard grass ill as a disease quite distinct from dysentery, louping ill, or "wool ball." On the other hand, it is similar to Braxy as it occurs in hogs. Sometimes chronic cases of dysentery, and occasionally recovered cases, may be affected with grass ill, which occurs as an acute intermittent disease and causes death. In two of our cases we discovered lesions of lamb dysentery, but we did not consider that the presence of these ulcers had any special significance, other than illustrating that lambs may be affected with a mild type of dysentery insufficient to cause visible symptoms or arrest development. From Cases L8 and L4 we were unable to isolate V. septique in culture, but it is important to note that none of the culture tubes from these two cases showed a B. welchii type of organism.

Our observations in the field and subsequent experimental work led us to believe that grass ill in lambs is caused by invasion of the system with an organism which produces a powerful toxin, and that this toxin causes certain characteristic lesions. The post-mortem picture is one

of toxæmia produced by an organism which we have found indistinguishable from *V. septique*. Examination of sections cut from the inflamed small intestines of affected lambs killed for post-mortem show enormous numbers of bacilli invading the submucosa. This agrees very closely with the pathology of Braxy as described by Gaiger⁸, with the notable exception that in only one case did we find any semblance of a lesion in the fourth stomach. Gaiger obtained thirty cases of sheep affected with Braxy, and a naked-eye lesion in the fourth stomach was observed in nearly every case. Where bowel lesions were also found, the microscopic appearances were identical with those in the stomach, though present in less degree. Hamilton⁹, when discussing Braxy, says "too much emphasis, it seems to me, has been placed on the stomach lesion as an essential feature of the malady. In my experience the sloughing hæmorrhagic patches found on the mucous membrane of the fourth stomach in some cases are completely absent in others." Apropos the stomach lesion, it is worth recording that a careful examination of Case G, a gimmer which died from Braxy, failed to reveal evidence of inflammation of the fourth stomach. The small intestines, however, were intensely inflamed, and the invading bacilli had apparently reached the animal's system by penetrating the intestinal wall. It is probably immaterial, and without effect upon the course of the disease, whether the causal organisms gain entry to the tissues through the stomach wall or *via* the intestines. It is unfortunate that our cases are so few in number and therefore we cannot be certain on this point.

As yet we are unable to suggest what exciting or predisposing cause or causes pave the way for the pathogenic action of *V. septique* in grass ill. This organism is a constant inhabitant of the intestinal canal of herbivora, and in the case of Braxy it is thought that the ingestion of frosted food chills the abomasum and allows the causal organism to penetrate its wall. Severe white frosts occur during May in the border counties, but we are unable to opine whether this is an important factor in the causation of grass ill.

We were successful in reproducing typical *V. septique* infections in two healthy lambs, by the use of cultures obtained from natural cases of grass ill. A study of the lesions observed as the result of the feeding experiment shows that they were similar to those already noted in naturally occurring cases of grass ill. So far as we know, there are no previously recorded cases of *V. septique* infection having been produced by the oral administration of this organism.

CONCLUSIONS.

- (1) Grass ill in lambs is caused by an organism indistinguishable from *V. septique* (Pasteur).
- (2) Grass ill is sometimes confused with lamb dysentery, louping ill, and "wool ball."
- (3) *B. welchii* types of organism have no etiological significance in grass ill.

(4) From cases of lamb dysentery we have isolated Dalling's "L.D. Bacillus."

(5) With the exception of the acrolein test, we have confirmed Dalling's differentiation of the "L.D. Bacillus" and *B. welchii*.

(6) We have noted that "L.D. Bacillus" ferments raffinose and not sorbite, while *B. welchii* ferments sorbite and not raffinose. These reactions proved constant with our strains.

We desire to record our thanks for help received from farmers and shepherds during field investigations; to T. Dalling, M.R.C.V.S., who kindly supplied the antitoxins used in our experimental work; also to G. E. Stephenson, M.C., M.B., of this University, for assistance in pathological histology.

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ENDEMIC INFECTION OF GUINEA-PIGS WITH *B. ÆRTRYCKE* (MUTTON).

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AT autopsies on guinea-pigs dying from intercurrent diseases it was noticed that some of the animals had marked empyema of the gall bladder.

Bacteriological Examination.

On examination of such animals the causal organism was the same in all the cases.* In the large majority the terminal condition was a septicæmia, as the causal organism, besides being always found in the intestinal canal, was isolated in pure culture from the gall bladder, heart blood, liver, spleen, and urine.

All autopsies were performed immediately after death, and in some cases the animals were killed when moribund so that cultures could be made with a minimum risk of contamination.

Morphologically this organism resembled members of the colityphoid group and was an asporogenic, actively motile, Gram-negative

* Since the completion of this paper the causal organism in some cases which were indistinguishable on autopsy from those due to *B. ærtrycke* has been *B. enteritidis* (Gärtner). At present the guinea-pig stock is suffering from endemic infections due to both organisms.

bacillus. It did not produce indol in peptone water. It grew luxuriantly in broth and on agar and gelatine, and the growth resembled that of *B. coli*. Gelatine was not liquefied.

Its fermentation reactions were as follows :—

Acid and gas were produced with arabinose, mannite, dulcitol, mannose, lævulose, rhamnose (iso-dulcitol), sorbitol, maltose, xylose, glucose, and galactose.

There was no acid or gas production with salicin, glycerine, dextrin, inulin, starch, lactose, saccharose, adonite, amygdalin, erythritol, raffinose, and inositol.

These reactions were carried out in ordinary peptone water containing 1 per cent. of the substance to be tested. The peptone water and the solution of the latter in distilled water were prepared separately, and in double the concentration which was desired for the completed medium. The H ion concentration of both solutions was adjusted to pH 7.6. The former, in amounts of 5.0 c.c., was sterilised at 120° C. for 15 minutes in test tubes containing Durham tubes, whilst the latter was sterilised in bulk by filtration through a Seitz filter. To complete the medium 5.0 c.c. of the latter filtrate were added to each tube of peptone water. In those tubes in which fermentation occurred the pH was reduced in from one to three days to between 4.6 and 4.3. The incubation was carried out at 37° C.

In milk a slight degree of acidity was produced in the first three or four days, but afterwards this slowly gave place to a definite alkalinity.

Agglutinating serum was prepared from rabbits. This serum was tested *re* its agglutinating power with emulsions of different organisms prepared according to Dreyer's method (1920).

The following organisms were not agglutinated in 1 in 25 or in any higher dilution of the serum :—

B. typhosus, *B. paratyphosus* A, *B. enteritidis* (Gärtner), *B. ærtrycke* (Newport), *B. dysenteriae* (Shiga), *B. pullorum*, *B. gallinarum*, and strains V, W, X, Y, and Z of *B. dysenteriae* (Flexner). This serum, however, agglutinated *B. paratyphosus* B. to a titre of 1 in 1,500, *B. ærtrycke* (Mutton) to 1 in 25,000, and the homologous organism to 1 in 20,000.

By means of absorption of agglutinins and comparative complement fixation tests this organism was found to be *B. ærtrycke* (Mutton). The technique employed was the same as that described in a previous paper (Edington, 1924).

Hereafter in this paper where *B. ærtrycke* is referred to it is to be understood that the organism meant is a *B. ærtrycke* (Mutton).

Pathological Examination.

Gall Bladder. It was the condition of this organ which first drew attention to the endemic. In most cases the gall bladder appeared as a distended bag of pus. The walls were dull white and much thickened, but adhesions to neighbouring structures were rare. Occasionally a guinea-pig died from the infection without showing any obvious gall bladder lesion, *i.e.*, in very severe cases with rapid death. The

presence of the lesion was a sign of a subacute or chronic illness, for experimental cases showed that three to five days had to elapse before the lesion became obvious.

The bile showed increased viscosity early in the disease, and grew progressively turbid until it looked like pus. During the early turbid stage the bile contained numerous epithelial cells and but few pus cells. When definitely purulent its chief constituents were disintegrating pus cells and Gram-negative bacilli.

Sections of affected gall bladders demonstrated that the thickening of the wall was chiefly due to thickening of the mucosa. The epithelial lining showed marked differences even in the same animal. In some places it retained its usual columnar form, in others it was cubical or even flattened, whilst in many it had disappeared and left the wall in an ulcerated condition. The reticular tissue of the mucosa, which is normally meagre in amount, was abundant and densely packed with cells. The latter, where the epithelium was intact, consisted of some polymorphonuclear, a few small mononuclear, and very numerous large mononuclear, cells. The latter were of the large macrophage type so characteristic of many of the lesions present in typhoid fever in man. Where ulceration was present there was a marked increase in the number of polymorphonuclear cells. The muscle layer showed slight infiltration with cells similar to those found in the mucosa (*v. supra*).

When doing routine autopsy work on guinea-pigs an animal was occasionally found with a small contracted, and often distorted, gall bladder whose walls were definitely fibrotic. This suggested the possibility that it had once suffered from this type of empyema. In some cases this possibility was strengthened by finding *B. ærtrycke* in the bile and ærtrycke agglutinins in the blood.

Liver. The liver was invariably enlarged and the parenchymatous cells showed cloudy swelling. In some cases these cells contained an excessive amount of fat.

The most noticeable pathological feature which is so characteristic of this type of infection was the presence of areas of focal necrosis. These were usually quite small, averaging 0.5 mm. in diameter; they ranged from microscopic size to 6 mm. in diameter. The pathology of this lesion has been discussed elsewhere (Edington, (1929)).

Abscesses were occasionally noticed, and from these pure cultures of *B. ærtrycke* could be obtained.

Spleen. This organ was usually enlarged and congested. On section the Malpighian bodies were more widely separated from each other than is usual. This was due to a great increase in the amount of the splenic pulp. The latter was packed with cells, chiefly red blood corpuscles and large macrophages. The former were more marked in the acute and rapidly fatal, whilst the latter predominated in the subacute and chronic, infections. The macrophage cells were actively engaged in the phagocytosis of red blood corpuscles, leucocytes,

and the products of cellular disintegration. Many of them contained masses of blood pigment.

Areas of necrosis were a common occurrence, usually situated near the surface of the organ, and generally accompanied by an adjacent peritonitis.

Occasionally large clumps of bacilli could be detected throughout the splenic tissue, and these characteristically did not produce any cellular reaction.

Abscess formation was sometimes present, and in rare cases this was a marked feature, the spleen being greatly enlarged and studded with abscesses up to 1.0 cm. in diameter. Such a spleen may attain the dimensions of 8 cms. $\times$ 4 cms. $\times$ 3 cms., and weigh 21.0 grammes. This great enlargement can be appreciated when the average measurements of the spleen in a fully grown guinea-pig are considered, *viz.*, 3.5 cms. $\times$ 1.75 cms. $\times$ 0.45 cm., and weighing 1.4 grammes. These large spleens are usually strongly adherent to their surroundings, especially round their upper poles.

Intestines. In the acute and subacute cases the intestines, especially the small, were congested. Areas of focal necrosis could sometimes be detected in the aggregations of lymphoid tissue in their walls, *e.g.*, Peyer's patches. Ulceration such as is found in human typhoid was rarely seen.

In the chronic infections surprisingly little abnormal could be observed, at the most slight congestion. It is interesting to note that in 105 autopsies of human typhoid fever collected by MacCrea (1907) four of these had no demonstrable intestinal lesion. Many others—Du Cazal (1893); Beatty (1897); Chiari and Kraus (1897); Flexner (1900), etc.—have also reported this atypical feature in infections due to organisms of the typhoid group.

Lymphatic Glands. Pathological changes in the lymphatic glands were best seen in the mesenteric group. These were usually enlarged and their sinuses packed with large macrophages. Focal necrosis and even abscess formation were sometimes discovered.

In some cases no lesion could be detected, and this was invariably in those animals in which no intestinal lesion could be found.

Blood. The blood in uncomplicated cases always showed a marked leucopenia. The leucocytes numbered between 2,000 and 4,000 per c.mm. The differential count was rarely much changed, but, if so, tended to an increase in the mononuclear cells.

As the disease progressed a diminution in the hæmoglobin percentage and red cell count was noticed.

The causal organism could frequently be recovered from the blood.

Lungs. Congestion in the acute cases and occasional patches of broncho-pneumonia or hypostatic pneumonia in the more chronic were the only pathological conditions detected.

Kidneys. Congestion and cloudy swelling were the usual lesions found, though an occasional nephritis has been recorded.

Urinary Bladder. A catarrhal condition of this organ was not infrequent, and the causal organism was often present in the urine.

Uterus. A purulent endometritis (Smith and Reagh, 1903) was sometimes present, and *B. ærtrycke* was isolated in pure culture from the uterine cavities of these cases.

Heart, Brain, Pancreas, Suprarenals, Ovaries, Testes. No pathological change other than congestion could ever be found in these organs.

Notes on the Infection.

The average number of guinea-pigs kept was 300, and of these about 60 were killed weekly, with, of course, a fresh influx of another 60 to take their places. The majority of these were used in the examination of milks for the presence of *B. tuberculosis*. They were of varying ages, none younger than three months, and were not bred on the premises. They were obtained weekly from one dealer, and were at first kept together in batches of 15 in large cages. These animals were removed, as occasion demanded, for inoculation purposes, and were therefore kept in pairs in smaller cages and in another room. The number of deaths from this infection during the period of one year was 171, and the monthly distribution of these was as follows: January, 7; February, 9; March, 17; April, 6; May, 9; June, 22; July, 19; August, 20; September, 21; October, 16; November, 11; December, 14. In only ten cases did both animals in the same cage die from this infection. In a series of 100 apparently healthy guinea-pigs *B. ærtrycke* was isolated from the intestinal tracts of three and from the livers and spleens of eight, whilst in 21 the blood serum agglutinated *B. ærtrycke* to a titre of 1 in 25 or higher. The highest titre obtained was 1 in 3,000.

Of the three animals which had *B. ærtrycke* present in their intestinal tracts two had *ærtrycke* agglutinins in their blood whilst the other had not. To demonstrate that guinea-pigs could harbour virulent *B. ærtrycke* in their intestinal tracks without any *ærtrycke* agglutinins appearing in their blood serum, six guinea-pigs were fed on food heavily impregnated with broth cultures of a virulent strain over a period of a month. Two of the animals died from an acute *ærtrycke* infection, but the others appeared healthy when killed at the end of the experiment and had no *ærtrycke* agglutinins in their blood sera.

In one of the eight cases in which *B. ærtrycke* was isolated from the liver and the spleen no *B. ærtrycke* could be found in the intestinal tract nor could *ærtrycke* agglutinins be demonstrated in the blood. This has been shown by Topley (1929) to occur in mice which have been infected with this organism, and it was this which gave me trouble in another investigation (Edington, 1929) which entailed the ligation of the common bile duct in guinea-pigs. Before operation all the animals had their fæces examined for *B. ærtrycke* and allied organisms, and their blood sera for agglutinins of these organisms. In spite of this a certain number died soon after the operation from acute *ærtrycke* infection. It would appear that the disturbance produced in the liver as well as in the general metabolism had allowed

the latent organism to burst into activity. One of the most interesting features in connection with diseases produced by *B. ærtrycke* and allied organisms is the method by which natural infection is produced. Different strains of *B. ærtrycke* vary greatly in their virulence for guinea-pigs, and this virulence is said to diminish rapidly when the organism is subcultured on agar. That this is not always so is shown by a strain which has been kept alive by weekly subculture on agar for over a year. The strain has been kept in the incubator at 37° C., and, though never of high virulence, is now as virulent as it was when first isolated. Strains showing a rapid decline in virulence are usually those which are particularly virulent when first isolated.

It is commonly accepted that the disease is readily produced in guinea-pigs by subcutaneous, intraperitoneal, or intravenous inoculation of a virulent strain, but that it is exceptionally difficult to produce the disease by feeding even massive doses of such an organism. Virulent organisms of the paratyphoid group may occur in the intestines of healthy animals over long periods, but what initiates the disease is not well understood. A large number of deciding factors have been suggested, and these will now be discussed *in seriatim*.

1. Onset of Another Disease.

This acts presumably both by lowering the resistance of the animal and also by injuring some organ such as the intestine, and so making a breach through which the organism can enter the system. The best example of this is afforded by hog cholera, of which the virus is the primary infecting agent and produces the lesions which make the animal susceptible to secondary infection with such organisms as *B. suispestifer* and *B. suissepticus*. In a *B. ærtrycke* enzootic amongst guinea-pigs at the Lister Institute Petrie and O'Brien (1910) attempted to prove that the primary cause was a filter-passing virus whilst the *B. ærtrycke* infection was of secondary occurrence. They had the usual difficulty in producing the infection by feeding recently isolated virulent strains of *B. ærtrycke*. Their experiments were unfortunately by no means convincing.

Meyer (1928) states that he has repeatedly tested and always failed to substantiate this view.

Though this investigation only entailed a study of an endemic infection, an attempt was made to infect guinea-pigs by subcutaneous, intraperitoneal, and intravenous injections of filtered saline extracts obtained from the intestines, livers, and spleens of animals dying from this infection (Seitz filters were used). In no case did any of the guinea-pigs so treated show any signs of ill-health. When this treatment was applied to animals which were being fed with virulent cultures of *B. ærtrycke* no increase in the number of infections over those occurring in the control animals was noted, except in the case where the injections were given intraperitoneally. Here, as will be shown later, the injection material probably acted not as a specific but as an irritant.

On account of the similarity of the technique to that just described the question of the importance of aggressins was next investigated.

Artificial and natural aggressins were prepared and administered orally, subcutaneously, intraperitoneally, and intravenously to guinea-pigs which were being fed on emulsions of virulent *B. ærtrycke*. No increase in the number of infections was noted except in the case of the intraperitoneal method of administration. In this it is probable that the material acts as an irritant.

(To obtain the aggressins from solid organs the livers and spleens of animals suffering from *B. ærtrycke* infection were ground up and extracted with a normal saline solution. The organisms were then removed by filtration through a Seitz or Berkefeld filter, or were killed by treating the saline extract with toluol. Serum exudates and fluid cultures were used as such after the organisms had been removed or killed.)

2. *Exposure of the Animals to Chills and Exhaustion.*

I have noticed that guinea-pigs which have just arrived after a railway journey during which they have been exposed to varying atmospheric conditions, and have been irregularly or perhaps never fed, have a higher incidence of infection than guinea-pigs living in the animal house under more equable conditions.

3. *Deficient Diets.*

Whilst commencing an investigation of the statement by Rénon (1914) that decrease in resistance to tuberculosis results on a vitamin poor diet I was preparing two batches of guinea-pigs on diets deficient in vitamins A and D (this was before the separation of A and D could be effected), and in vitamin C, respectively. These animals were kept in the same room as the stock animals from which they were originally drawn. The stock was at that time suffering from an endemic *B. ærtrycke* infection with a 5 per cent. mortality. Amongst the animals on the deficient diets the infection became epidemic and the mortality rose to 80 per cent. in the case of the vitamin A and D, and to 70 per cent. in the case of vitamin C, deficient animals. Death was considered due to *B. ærtrycke* infection when that organism was isolated from the heart blood.

The diets were as follows, to produce :—

- (a) Vitamin A and D deficiency, the diet used by Tozer (1921).
- (b) Vitamin C deficiency, that used by Finlay (1923).

Remarks on the Effect of the Various Vitamins.

Vitamin A.—It has been recognised for some time that deficiency of this vitamin in the diet produces a train of sequelæ amongst the chief of which is the proneness of the animal to the development of infection. This infection is sometimes caused by organisms which are avirulent to the healthy animal.

The method of action is not fully understood; but from the researches of Mori (1922), Goldblatt and Blüschel (1927), Cramer (1923, 1924), and others, it would appear that some of the important factors are the metaplasia of columnar, transitional, and cubical epithelia to the squamous variety, and the marked changes in the villi and mucus-secreting cells of the intestines, *viz.*, atrophy, necrosis,

and invasion by organisms. The defective mucus secretion is also thought to play an important part, and this is supported by the contention of Bond (1921) that mucus plays a biochemical as well as a mechanical part in the protection against infection.

In experimental work Werkman (1923) has clearly shown that if white rats and rabbits are subjected to a vitamin A deficient diet their resistance is lowered to infection by *B. anthracis*, and by an organism, resembling the pneumococcus, which he had isolated from the pleural cavity of a moribund rat.

On the other hand, a diet containing an ample supply of vitamin A is considered a guard against infection. Indeed, Green and Mellanby (1928) were so struck with its efficacy in this respect that they called it the "anti-infective" vitamin, and have more recently (Mellanby and Green, 1929) used it with success in concentrated form and by oral administration in cases of puerperal sepsis due to the hæmolytic streptococcus.

Experimentally, Pritchett (1927) showed that in mouse typhoid (*B. pestis caviæ*) the resistance of the animals was raised by adding 5 per cent. of butter fat or cod liver oil to a bread and milk diet which was in itself adequate to promote the breeding and rearing of a healthy stock through a period of years. A similar effect was obtained with a McCollum "complete" diet even when the butter fat was omitted, and also with a bread and milk diet in which the milk had been rayed with a mercury lamp. This statement is interesting in view of the controversy regarding the efficacy of vitamin D as an anti-infective agent.

Vitamin B.—Animals on a vitamin B deficient diet also show a greater liability to infection. This was demonstrated by Guerrini (1921) in the case of pigeons, and later by Werkman (1923) in pigeons and rats. This deficiency produces an atrophy of the lymphoid tissue, and this, according to Cramer, Drew, and Mottram (1921), is the cause of the general marasmus and fall of temperature. These effects of a vitamin B deficient diet have probably a bearing on the lowering of the animal's resistance to infection.

Vitamin C.—A diet deficient in this vitamin also renders an animal more liable to infection. This has been shown by Finlay (1923) in guinea-pigs, which were found to be less resistant to *B. coli*, *Streptococcus hæmolyticus*, *Straphylococcus aureus*, and the *Pneumococcus*. He found degenerative changes and feeble leucoblastic reaction in the bone marrows of animals suffering from a deficiency of this vitamin, and stated that these may be factors in the reduction of resistance to bacterial infection.

Werkman, Nelson, and Fulmer (1924) have also demonstrated the effect of this deficiency in lowering the resistance of guinea-pigs to *B. anthracis* and the pneumococcus.

Vitamin D.—There is a difference of opinion regarding the importance of this vitamin in the prevention of infection.

Green and Mellanby (1928) consider its action negligible, whilst Eichholz and Kreitmair (1928) found in their experiments

on rats that a lowered resistance was produced to infection with paratyphoid and a virulent strain of the pneumococcus.

In summarising the above, it appears that to keep an animal in as resistant a state as possible to infection a well balanced diet containing an ample supply of the various vitamins is essential. We have found this in the case of our guinea-pig stock, where the lack of their usual ration of green food increases their mortality from intercurrent infections.

4. *Injury to the Intestinal Tract.*

This appears to be one of the methods by which a vitamin A deficiency acts in producing an increased liability to infection (*v. supra*).

By feeding guinea-pigs with small quantities of ground glass and emulsions of a virulent *B. ærtrycke* a high incidence of infection (85 per cent.) has been obtained. Amongst the controls the corresponding figure was 25 per cent.

By injecting an irritant, either mechanical, chemical, or organismal, into the peritoneal cavities of animals being fed on virulent *B. ærtrycke*, it has been possible to raise the incidence of infection. (45 to 80 per cent. as compared with 25 per cent. amongst the controls). Some of the irritants used were suspensions of ground glass and fine sand, weak solutions of hydrochloric and sulphuric acids, and emulsions of slightly virulent organisms, *e.g.*, *B. coli*.

This is the probable explanation of the previously mentioned action of the intraperitoneal administration of tissue and bacterial extracts.

I also noticed in another investigation, which entailed the oral administration of rather large quantities of soaps and fatty acids to guinea-pigs, that many of these animals succumbed to *B. ærtrycke* infection. It is suggestive that the chief post-mortem feature of animals so fed was a remarkable atrophy of the intestinal walls. Again, when the common bile duct of a guinea-pig is ligated and the animal is fed on virulent cultures of *B. ærtrycke* the number of infections produced is exceedingly high. Such animals commonly have a catarrhal, sometimes even amounting to an inflammatory, condition of the duodenum and upper part of the jejunum.

This is not due to the action of the organism, as it occurs in control animals, and is probably caused by the digestive disturbance resulting from the ligation. This intestinal lesion is a possible point of entrance of the infection. I do not wish to stress unduly this possibility, as there are obviously other factors to be taken into account, *e.g.*, derangement of metabolism and possible mechanical injury to the peritoneum and intestine resulting from the operative procedure.

Webster (1923¹) has shown that bile administered *per os* to mice in maximum doses tends to increase their susceptibility to mouse typhoid. He explains this by quoting Besredka's statement (1919) that bile, if administered in sufficient doses by the mouth, injures the intestinal epithelium and allows pathogenic organisms easy access to the blood stream.

5. *Virulence of the Organism.*

The importance of this in the initiation and spread of an epidemic of mouse typhoid has been questioned by Webster (1923²), who concluded that virulence was a constant factor.

This has been opposed by Lockhart (1926), who stated that fluctuations in virulence do occur (B. *ætrycke* infection in mice was studied in this investigation), and that virulence should not be disregarded in considering the spread of an epidemic.

In observing the result of feeding B. *ætrycke* cultures to guinea-pigs one cannot help acquiring the impression that changes in virulence play a relatively unimportant part in the production and spread of the infection.

In our present state of knowledge a reasonable view appears to be that of Smith and Nelson (1927), who consider that the passage from the epidemic to the endemic state is accomplished by the death of animals of low natural resistance and the gradual adjustment of the organism to the population on a lower level.

In amplification of this I think the passage from the endemic to the epidemic might well be considered as due to an influx of animals of low natural resistance accompanied by the further lowering of the herd resistance by the introduction of such factors as deficient diet, intestinal injuries, onset of other infections, etc. (*v. supra*), and to a gradual rise in the virulence of the organism.

SUMMARY.

An endemic due to B. *ætrycke* (Mutton) has been investigated. The causal organism and the pathological lesions which it produces in guinea-pigs have been studied.

Various methods by which the infection can be produced have been mentioned, and their probable bearing on the mode of natural infection indicated.

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A NOTE ON THE MERCURIC CHLORIDE TEST FOR TRYPANOSOMIASIS.

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OUR experiments on this test were carried out during March of this year, and were based on the successful method adopted by Bennett¹ when using this drug as a diagnostic for camel trypanosomiasis.

The test was carried out on cattle, goats, horses, and pigs.

Our tests on cattle were carried out on sera from nine controls, two congolense cases, three vivax cases, and two Theileria cases.

The mercuric chloride was used in various dilutions and proportions with sera, namely, 1 c.c. of 1 in 20,000 mercuric chloride + 1 drop serum; 1 c.c. 1 in 30,000 mercuric chloride + 1 drop serum; 1 c.c. 1 in 30,000 mercuric chloride + 1 drop serum and aqua dist. aa.; 1 c.c. 1 in 20,000 mercuric chloride + 1 drop serum and aqua dist. aa.; 1 c.c. 1 in 40,000 mercuric chloride + 1 drop serum in various dilutions.

In all cases when cattle sera were used there was no appreciable difference between controls and infected, opacity occurring in all tubes.

We therefore conclude with Hornby² that the test is useless as a diagnostic for cattle trypanosomiasis.

Both normal and infected goat sera also precipitate the solution.

A few tests carried out on horse's sera and pig's sera gave some promising results, but further tests should be carried out on more suitable animals before any conclusion can be drawn.

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PARAGANGLIOMATA OF THE ADRENAL MEDULLA, WITH A REPORT OF THREE CASES IN CATTLE.

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Endocrine tumours as a group are especially interesting, not only for the intrinsic features of the growth but also because the neoplasm frequently contains the active secretion of the gland in which the growth originated. Of these tumours, the paraganglioma of the adrenal medulla is both complex in structure and in origin; and in most cases it contains adrenalin, the essential secretion of the adrenal medulla.

The paraganglioma was first described by Manasse, in 1896, and since then it has been variously termed a chromaffin tumour, struma medullaris suprarenalis, paraganglioma, and phæochromocytoma. In all, thirty cases of this adrenal tumour in the human subject have been described and the growth has been thoroughly investigated, but few descriptions of paragangliomata in the adrenals of animals are available, and for this reason, chiefly, the following three tumours occurring in cattle were investigated in detail.

According to Stilling, quoted by Berdez, they are more common in animals than in man, but in spite of this we have not been able to find many recorded cases. Zanfrogini has described a pigmented tumour in the adrenal of a cow. Alezais and Peyron, and Peyron, in an extensive study of the subject described several in horses and one in a sheep, and refers to their occurrence in cattle. In one case in a horse the growth was malignant. Ameuille described a chromaffin tumour in a horse. Kimura described two in horses and stated that altogether ten in horses and four in cattle had been recorded.

In man, the tumour generally has given rise to no particular symptoms, although Orth, Vaquez, Oberling, and Jung have described cases in which arterial hypertension co-existed. Neurofibromatosis was associated in cases recorded by Suzuki, Kawashima, Herxheimer, and Zeckwer. Of the thirty human cases collected by Rabin, eighteen were females and six males; thirteen occurred in the right gland, and nine in the left. Only six were bilateral. Neusser and Wiesel have described a tumour in a child of two years, but apart from this the recorded cases are of persons over forty years of age. The tumour may vary from the size of a pea to about ten cm. in diameter, is usually well encapsulated, and occupies the whole or greater part of the medulla. The cross section varies in colour from a greyish red to a reddish brown, and is of soft and cellular appearance. The growth consists of large epithelium-like cells in an alveolar grouping,

in strands, or in large masses separated by fine connective tissue fibrils, coarse fibrous septa, anastomosing capillaries, or large blood sinuses. The cells are round, polyhedral, or spindle-shaped, and possess a round, oval, or vesicular nucleus. Giant cells with single or multiple nuclei are not uncommon. The cytoplasm is finely granular, vacuolated, and may contain acidophilic inclusion bodies.

Like neoplasms arising from the sympathetic nervous system, the adrenal paraganglioma may include cells of sympathetic origin in the various stages of differentiation. Herxheimer found medullated and non-medullated nerve fibres, and Hedinger non-medullated nerve fibres and ganglion cells, while Hedinger, Marchette, Suzuki, Wegelin, and Landau describe tumours containing sympathetic formative cells. Hedinger, Suzuki, and Wahl have described paragangliomata combining the typical features of the ganglioneuroma, the neuroblastoma, and the paraganglioma within the one growth.

The available descriptions of chromaffin tumours in animals suggest that the appearances in general are similar but more frequently uniform. In both the human and animal tumours adrenalin may be present. (Hedinger, Orth, and Wagelin).

The recognition of chromaffin cells is based on the presence of the chrome-reacting cytoplasmic granules, and these granules are believed to be associated with the presence of adrenalin. From the cortical cells the chromaffin cells are readily distinguished by the absence of fats and lipoids in the latter. In sections the cytoplasmic granules reduce gold chloride, and with ferric chloride the cells turn a greenish colour. When treated with chromates medullary tissue assumes a brown colour, while formalin fixed tissue shows a special affinity for hæmatoxylin (Swale Vincent and Goldzieher) and for saffranin (Swale Vincent).

In an investigation of tumours of the adrenal medulla some reference to the development of the adrenal medulla is necessary. In mammals and man the development is similar (Sharpey-Schafer) except that the chromaffin substance appears after birth in man, and long before birth in the ox and sheep (Bruni—quoted by Swale Vincent).

In the human embryo the medulla arises from a group of cells at the side of the aorta which also form sympathetic ganglia. The medullary cells begin to migrate into the cortex during the eighth week, and this continues throughout the greater part of fetal life. By the fourth month they are arranged in reticulating columns on the walls of branching venous sinuses. Gradually the cells increase in size, chiefly through acquiring more cytoplasm, and simultaneously the cytoplasmic granules develop. When the cells acquire these chromaffin reacting granules the mature form is complete.

From the standpoint of tumour development we may therefore expect either a neuroblastoma originating from the primitive sympathetic neuroblast or either of two forms representing the specialised tissues which may result from the primitive medullary cells; that is, the ganglioneuroma which has developed along the lines of the sympathetic ganglia, or the chromaffin tumour which consists of tissue resembling that of the adrenal medulla. As shown in the literature, features of all these

types of growth may occur in the same tumour. In few cases do the paragangliomata consist solely of chromaffin cells.

After this preliminary survey, the findings in the following three cases will be interpreted more readily.

The three cases were discovered during the routine examination of carcasses at the Birkenhead lairages, by one of us (H.L.T.). In two cases the other organs showed no naked-eye abnormality, while in the third there was a malignant hepatoma of the liver. In two cases the tumours were unilateral, in the third bilateral.

Case I. (28-382). Irish Bullock of about three years. Unilateral.

A cross-section of the adrenal was round; and a much thinned yellowish cortex encircled a medullary tumour of a dark reddish-brown colour and about the size of a Jaffa orange. The cortex was sharply differentiated from the tumour. The neoplastic tissue was extremely vascular and very friable.

Histological examination revealed that the cortex was thin, but showed no actual invasion. The growth was well encapsulated and consisted of polyhedral and spindle-shaped cells of about 6 by 12μ and with a nucleus of about 5μ . The cytoplasm was finely granular and frequently vacuolated. The cells were grouped into acinar bundles or large masses, and these groups were separated usually by fine connective tissue fibrils, but in places by coarse fibrous septa. Scattered throughout were small masses of round cells, but, as they chiefly occurred in the fibrous septa, they were considered to be lymphocytes. In general the growth was well vascularised by large endothelium-lined spaces (Fig. 1.)



FIG. 1.

Case I.—The tissue is made up of round and polyhedral cells with occasional spindle cells intermixed. This area shows the characteristic thin-walled blood sinuses and one fibrous septum. ($\times 100$.)

Pieces of tissue that had been fixed in 10 per cent. formol saline showed an intense bluish staining of the protoplasm, in contrast to the pale staining of the cortical cells. The material used for the chrome reaction had been

in 10 per cent. formal saline for three days prior to being placed in the solution of a chrome salt (Helly's Fluid), and because of the initial formalin fixation a chrome reaction did not appear to be feasible. Blocks of tissue treated in this way, however, when sectioned showed a fairly even brown staining of all the tumour cells which sharply differentiated them from the adjacent cortical cells. On high power examination the cytoplasm of the brown-staining cells appeared rich in fine granules.

Case II. (29-336.) Old Irish Cow in poor Condition. Unilateral.

The adrenal was swollen to an oval form owing to the presence in its centre of a tumour measuring 2.5 cm. in diameter (Fig. 2). The cut surface showed the cortex to be much thinned. It varied from 3 mm. to 1 mm. in thickness. An outer band of cortex was a bright yellow, but merged into a dull brownish colour at the border of the tumour. The tumour was well demarcated from the cortex, and in colour varied from a dull brown at the periphery to a yellowish colour at the centre. Some coarse lobulation of the growth was evident. Hæmorrhage had occurred in several areas.

Microscopic examination showed some evidence of pressure-atrophy of the cortex. The tumour was well encapsulated. Its cells in many areas were arranged in acinar groups bounded by fine connective tissue fibrils. Almost as frequently, however, the cells were in large masses. On the whole, there were few blood sinuses but many capillaries vascularising the growth.

The tumour cells were round, about 8μ in diameter, and contained a granular nucleus of about 5μ in diameter. The chrome and hæmatoxylin reactions were distinctly positive. Sections stained with Sudan III showed the absence of fats and lipoids in the tumour tissue, both of which were present in the cortex.

The liver in this animal contained a primary carcinoma associated with a distinct cirrhosis. The cells were distinctly of hepatic origin and were not related to the chromaffin cell.

Case III. (29-365.) Aged Irish Cow. Bilateral.

Right Adrenal: The superior fold was of about normal size, but from this point the cortex was thinned out as it encircled the tumour. Over the paraganglioma the cortex was about 2 mm. in thickness. The tumour measured

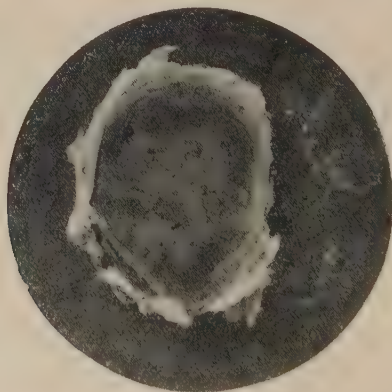


FIG. 2.

Case 2.—The thin layer of cortex encircling the growth can be distinguished. The medullary tumour shows some coarse lobulation at its centre and a few hæmorrhagic areas. (Actual size.)

about 15 by 9.5 by 11 cm., and was roughly divided into an upper portion 8 by 9.5 cm. and a lower portion about 7 by 9.5 cm. The upper division was of a light brown colour, with only an occasional reddish area, and showed irregular lobulation. The aorta was lightly adherent and gave no evidence of invasion. The lower division of the tumour was a light brown, mottled with red, and contained coarse fibrous septa. This area was extremely vascular. (Fig. 3.)

Histological examination showed that the cortex was thinned and had been invaded in a few areas. The tumour cells were in large masses with scanty stroma, but on the whole there was an abundance of blood sinuses. Coarse fibrous septa were irregularly distributed. The cells were chiefly round or polyhedral, about 10 by 6μ , and contained a round granular nucleus of about 4μ in diameter. The cytoplasm was granular and frequently vacuolated.

In the lower portion of the growth the cell masses frequently had eosinophilic protoplasm and the cells were of lesser dimensions, closely packed together, and with indistinct outlines which gave a syncytial effect. Among these eosinophilic cells others showing the typical chromaffin characteristics were interspersed, and many areas contained numerous transition forms between the two types. Stroma, generally, was scanty and blood vessels were scarce. There were many large areas of necrosis. The cytoplasm of these eosinophilic tumour cells was granular and frequently globulated. Scattered irregularly through this section of the tumour were spaces of varying sizes, caused by degeneration.

Sections stained with Sudan III showed a total absence of fat within the neoplasm. Both the chrome and hæmatoxylin reactions were moderate but distinct.

Left Adrenal: The gland was round in shape and contained a central

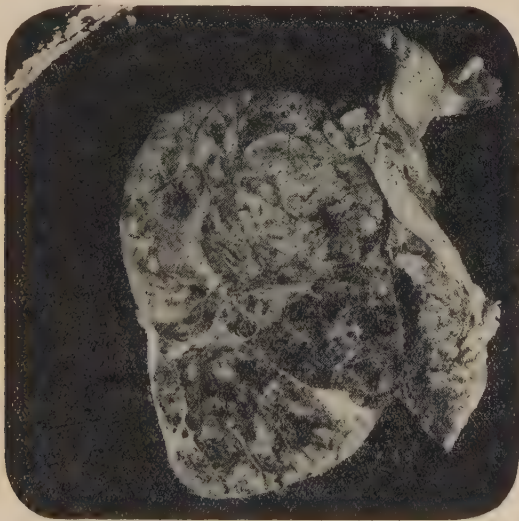


FIG. 3. $\times$ one-third. Case 3.

Case 3.—The cortex cannot be identified readily. The paraganglioma of the medulla is roughly divided into an upper and a lower division. The whole growth contains numerous coarse septa. In the upper division dark mottling of the neoplastic tissue predominates, while irregular lobulation is more evident in the lower portion. (One-third actual size.)

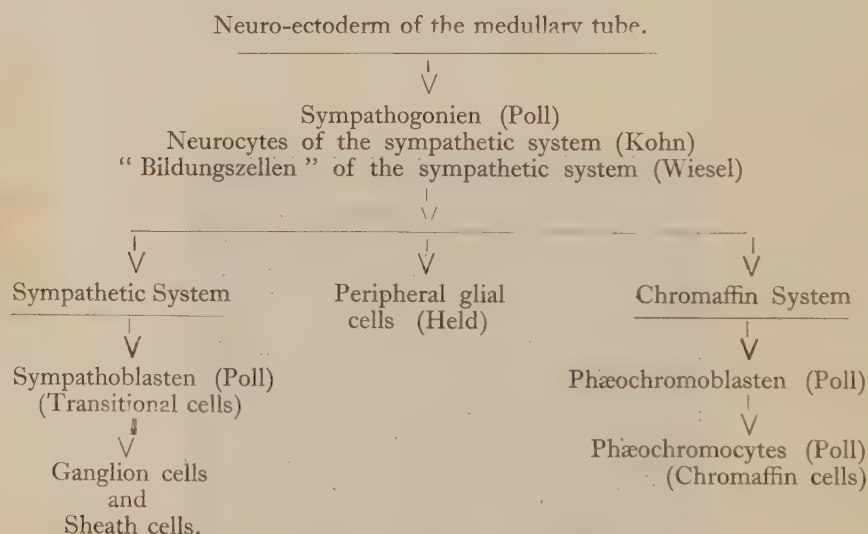
soft growth 9 by 5 by 11 cm. which was well encapsulated and sharply demarcated from the cortex. Encircling the tumour was a thin light brown band of cortical tissue which varied from 0.5 to 0.2 cm. in thickness.

Sections of the neoplasm showed that the tissue was well supplied with thin fibrous septa between which were masses of round and polyhedral cells that stained intensely with hæmatoxylin. Neither necrosis nor hæmorrhage had occurred. In general, the appearances were similar to those of the growth in the right adrenal except that no eosinophilic epithelium-like cells were seen.

DISCUSSION.

Of the three animals, two were females, and one male; all had reached the adult stage, but their actual ages could not be ascertained. Two showed unilateral and one bilateral tumours of the adrenal medulla, and in only one animal were there any lesions in the remainder of the organs.

The situation and characteristics of the growth described show them to be paragangliomata of the adrenal medulla. This is confirmed by the histological characteristics of the cells and the chrome reaction. The origin of tumours of the adrenal medulla is well indicated in the scheme of Poll and Landu as modified by Wahl.



In the tumours described the structure is that of the matured paraganglioma except in Case III. The eosinophilic epithelium of Case III. may on the one hand be considered as chromoblasts (phäochromoblasts); but the general impression of the microscopic features growth seems to indicate that they are closely related to the adult chromaffin cell, and more especially so because a number of these eosinophilic epithelium-like cells were frequently scattered in tissue otherwise typical of a paraganglioma. These eosinophilic cells, therefore, may represent a modified form of the adult chromaffin cell.

Definite figures relative to the incidence of paragangliomata in cattle are not possible, as all the animals slaughtered within the Landing Place are not subjected to a routine examination. However, of 73,244 apparently healthy cattle slaughtered and examined in 1928, only one (Case I) had a tumour of the adrenal medulla.

The two remaining cases belong to another group. In 1929, up to November 1st, 108 cattle were slaughtered because of injury in transit or for emergency reasons. Such animals in all cases undergo a complete post-mortem examination. Of the 108 carcasses examined, two showed adrenal paragangliomata (Cases II and III).

To Professor J. Shaw Dunn we express our thanks for the interest he has taken in this work.

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A NOTE ON BOVINE TUBERCULOSIS IN TROPICAL AFRICA (BRITISH CAMEROONS).

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THE following is an extract from a report on a visit during February of this year to Buea, in the Cameroons Province, in order to investigate the reported occurrence of tuberculosis amongst the cattle on a Stock Farm. The investigation was carried on at the instigation of the Chief Veterinary Officer, Captain W. W. Henderson.

The farm is held by a German Company, the West African Plantations Limited, of Victoria, and is situated on the Eastern slopes of the Cameroons Mountains at an altitude of approximately 3,500 feet. Ample grazing is available throughout the year.

The function of the farm is the supplying of meat, milk, and butter to the Europeans living in the vicinity of Buea and Victoria.

There are two main farm buildings, one in which the main milking herd is housed and the other in which are housed the five pure bred imported Algauer cattle and the young stock. From a hygienic and sanitation point of view the construction of the buildings and their arrangement are very good. There was an efficient drainage system, and an excess of air space, but the arrangement of the animals in the main milking herd was not good, since they were in two rows facing each other. These animals are grazed during the day, but housed at night. The pure bred imported cattle are housed at all times.

For purposes of the tuberculin test the cattle were divided into two sections. The first batch tested consisted of the twenty-five animals in the main milking herd made up of one pure bred imported Zebu cow, two pure bred Algauer cows, and 22 cross breeds, either cross between Zebu and native or cross between Algauer and native cattle. The second batch consisted of two pure bred imported Algauer bulls, three pure bred Algauer cows, and the yearlings, calves, work oxen, and two cows which had previously been sold off the farm to natives in the vicinity of Buea. All calves are separated from their mothers immediately after birth.

The test was carried out by the subcutaneous method. Materials were not available for the intradermal test. In all cases temperatures were recorded at the following times :—

Pre-Inoculation Temperatures :

1st day, 8 a.m., 5 p.m.

2nd day, 8 a.m., 5 p.m., and 11 p.m.

3rd day, 5 a.m., 8 a.m., 11 a.m., 2 p.m., and 5 p.m.

Post-Inoculation Temperatures :

1st day, 11 p.m.

2nd day, 5 a.m., 8 a.m., 11 a.m., 2 p.m., 5 p.m.

If considered necessary, further readings were taken at 8 p.m. on the second day and 8 a.m. on the third day.

A rise of 2.7° F. or over was considered to be a positive reaction. An increase of temperature of over 1.4° F. and less than 2.7° F. was considered to be a suspicious reaction.

The above are the diagnostic reactions given by the Institute Pasteur, who issued the tuberculin used.

The doses injected were as follows: Bulls and oxen 4 c.c., cows 3 c.c., yearlings 1.5 c.c., and calves 1 c.c.

Of the twenty-five animals in batch one the following gave positive reactions:—

- | | | |
|-----|-----------------------|---------------|
| 1. | Cross bred cow | 15 years old. |
| 2. | " " " | 6 " " |
| 3. | " " " | 4 " " |
| 4. | " " " | 4 " " |
| 5. | " " " | 5 " " |
| 6. | " " " | 8 " " |
| 7. | " " " | 6 " " |
| 8. | " " " | 4 " " |
| 9. | Half bred Zebu cow | 8 years old. |
| 10. | " " " | 12 " " |
| 11. | Pure bred Algauer cow | 9 years old. |
| 12. | Pure bred Zebu cow, | aged. |

This gave the very considerable average of practically 50 per cent. reactors in the herd consisting mainly of cross bred cattle living under extremely good hygienic conditions.

Of the second batch of thirty-one animals, two reacted, both being imported pure bred Algauer bul's three years and six months old. These two animals had passed the test in Germany two years ago.

None of the calves or yearlings reacted, nor did the two animals which had been previously sold off the farm to natives. One ox and one pure bred imported Algauer cow gave doubtful reactions. These were therefore re-tested by the method of Vallée, but did not react.

The total percentage of reactors was twenty-four.

The high incidence of reactors is of additional interest in view of a similar result recorded by Purchase* in a herd in Northern Rhodesia.

The fifteen-years-old cross bred cow, number 1 above, was slaughtered, and post-mortem revealed an area the size of a saucer near the apex of the right lung, showing typical tuberculous lesions. On section numerous caseous foci were observed, with proliferation of the connective tissue. The bronchial glands were considerably enlarged and hæmorrhagic. The supra-mammary glands were indurated, but no macroscopic lesions of tuberculosis were observed.

Smears from the lesions revealed the typical acid-fast causal organism.

Three guinea-pigs were inoculated with an emulsion from the

* The Incidence of Tuberculosis in a Herd in Northern Rhodesia. *Journal of Comp. Path. and Ther.*, Vol. xlii, Part 2, June, 1929.

lesion, and in twenty-two days all showed enlargement of the inguinal glands. One was then killed, and the organism was recovered from the left superficial inguinal gland. The remaining two pigs died in 28 and 39 days, respectively, subsequent to inoculation, from generalised tuberculosis. Cultures of the organism are being propagated at the Laboratory.

One concludes that the disease was brought in several years ago by pure bred cattle imported from Germany. None of these cattle are now alive, but the cross bred herd so heavily infected consists mainly of their progeny.

In spite of the fact that these animals were housed under good conditions, grazed outdoors during the day, and were situated only a few degrees north of the equator, the spread of tuberculosis in the herd appears to have been very rapid.

Bovine tuberculosis can, therefore, quickly and heavily infect cattle in the tropics even when they are kept under good conditions, and animals should not be imported into the tropics unless they have previously passed a rigorous tuberculin test.

It might be of interest here to quote the following paragraph from the remarks made by Dr. Tertius Clarke* during a discussion before the Royal Society of Tropical Medicine and Hygiene, on a Paper, "Pulmonary Tuberculosis in the Tropics," by Dr. W. S. Hodson:

"Again the incidence of bovine tuberculosis came into consideration. In the tropics it was nearly absent. He had no record of any in 250,000 pigs examined under his supervision at the Ipoh abattoir during six years. He knew no veterinary surgeon who had seen the disease in cattle in Malaya. The only records he could find, apart from Kenya which lay very high, were in French possessions. In Dakar there was a herd with tubercle; in the South of Madagascar the Baras took milk largely from heavily infected cattle, but remained without infection. There was said to be bovine tubercle in adult cattle, but not in calves, in South Annam. In the Belgian Congo there was much. In thirteen British Colonies and dependencies in the tropics there was none reported apart from Kenya. He made, he said, no deductions from the figures he had cited."

I am indebted to Mr. G. E. Owen, M.R.C.V.S., Acting Chief Veterinary Officer, for permission to publish these notes.

* Discussion on Paper by Dr. W. S. Hodson. *Transactions of Royal Society of Tropical Medicine and Hygiene*, Vol. xxiii, No. 1, June, 1929, p. 37.

REVIEWS.

The Principles of Bacteriology and Immunity. By W. W. C. Topley, M.A., D.Sc., F.R.C.P., and G. S. Wilson, M.D., M.R.C.P., D.P.H. London: Edward Arnold & Co., 1929. Price 50s. net.

In the preface to this work the authors explain that in producing it they have had in mind the requirements not of the ordinary student, or even of the ordinary post-graduate student, but of those students of medicine and biology who wish to make a serious study of bacteriology and its application to the problems of infection and resistance, and it is submitted that the matter contained in the two volumes is of a kind with which any bacteriologist should be familiar. The inference from these and other statements in the work is that it is designed for those who aspire to have the right, from knowledge of the subject, to describe themselves as bacteriologists. It is admitted that it has a frank bias in the direction of the application of bacteriological principles to the study and control of infective disease, and for that reason numerous instances of natural disease in live-stock have been included in it.

In point of size no previous English text-book on bacteriology can compare with it. It extends to over 1,300 pages, and the greater space has allowed the authors to deal much more fully than is usual with some branches of the subject. About half of Vol. I is devoted to what is termed General Bacteriology, which deals in a general way with the biological characters of bacteria, while the remainder deals with Systematic Bacteriology and includes an account of the different species of bacteria in respect of their morphological, cultural, and staining characters, and their pathogenicity. In Vol. II the subjects are arranged under two main heads, viz.: (1) infection and resistance, and (2) the application of bacteriology to medicine and hygiene. An unusual feature of the work, and one that certainly has some disadvantages, is that the description of the organism and of the account of the disease for which it is responsible are not consecutive, but actually in different volumes.

In a work in which, under the authors' scheme, every aspect of the subject had to receive consideration, it is difficult to draw comparisons between different parts, but the opinion may be expressed that the last section is the one deserving most praise. It presents the reader with a well-balanced account of the infective diseases of the human species, and omits nothing that ought to be known to those whose work lies in connection with the prevention of these diseases in man. It must be admitted, however, that the same cannot be said with regard to the like diseases in the domesticated animals, the condensed references to those being inadequate for the purpose of a veterinary bacteriologist, and occasionally inaccurate.

The work includes 241 text figures, which are generally good, but an exception must be made of the two depicting the *Bacillus anthracis*, which, apparently owing to the method of staining, are misleading rather than helpful for diagnosis.

The publisher's share of the work deserves commendation.

Insects, Ticks, Mites, and Venomous Animals of Medical and Veterinary Importance. By Walter Scott Patton, M.B., CH.B. (EDIN), F.R.S., and Alwen M. Evans, D.Sc. Illustrated by Edith Mary Patton, Alwen M. Evans, and A. J. Engel Terzi.

This work belongs to the class of books that are most welcome to a reviewer, because he finds nothing in them that is not deserving of commendation. It has been written primarily for medical officers and others taking post-graduate courses in Tropical Medicine, and takes the place of Patton and Cragg's

Textbook of Medical Entomology, which was published sixteen years ago, and is now out of date and out of print. Its bulk (785 crown 4to pages) is apt to suggest that it must contain matter that is superfluous for the requirements of those for whom it is designed, but the authors have had in mind not only the medical graduate who is studying the subjects with a view to obtaining a diploma in Tropical Medicine, but also the medical officer stationed in the tropics, who is often cut off from access to a library in which he might find the information necessary for original research when insects or acari appear to be a factor in the dissemination of disease in man or animal. A striking feature of the work is the number (374) and excellence of the illustrations.

The volume has been published privately, and is only obtainable on application to the Department of Entomology, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool. Price 20s.

The Essentials of General Physiology. By Eric Ponder, M.D., D.Sc., Professor of General Physiology, New York University. London: Longmans, Green & Co., 1929. Price 15s. net.

What exactly should be included in a textbook on General Physiology is a matter about which the opinions of authors are not unanimous, and the scope of this book is not fully disclosed by its title. Its object is not to describe the functions of the different organs of the body, but to explain the phenomena underlying the vital processes in terms of known physical and chemical principles. The first four chapters deal exclusively with physical processes, viz.: surface action, the colloidal state, diffusion and permeability, and the dissociation of electrolytes. The consideration of these subjects occupies about a third of the book, and in the remaining chapters the principles thus laid down are employed to explain as far as possible the different vital phenomena—muscular action, digestion, respiration, secretion, etc. There are special chapters on enzymes, hormones, and fertilisation.

There can be no doubt that the work will form a valuable supplement to the textbooks on physiology that are in general use, and it can truly be said that practitioners as well as students will find it interesting reading.

The Essentials of Histology. By Edward Sharpey Schafer, F.R.S., Professor of Physiology in the University of Edinburgh. Twelfth Edition. London: Longmans, Green & Co., 1929. Price 15s. net.

Since its first appearance in 1885, Professor Schafer's textbook has been in the front rank of English works on Histology. In preparing the new Edition the text has been carefully revised by the author, with the assistance of Dr. H. M. Carleton, Lecturer on Histology in the University of Oxford, and numerous new illustrations have been introduced. It presents the essential facts in adequate detail for the medical student, and in language that is clear and concise. Its authority on histology can scarcely be challenged, but it is surely a slip to say (p. 44) that the large uninuclear leucocyte "is the most phagocytic of white cells, especially towards foreign bodies and parasite organisms."

Bainbridge and Menzies' Essentials of Physiology. Sixth Edition. Edited and revised by H. Hartridge, M.A., M.D., Sc.D., F.R.S. London: Longmans, Green & Co., 1929. Price 14s. net.

The fact that the present is the sixth edition since 1914 proves that this textbook is well adapted to the needs of students of physiology. The revision and addition of a number of new chapters, including one on basic principles, have brought the work completely up to date.